MESOCOSM STUDIES: POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) EXPOSURE, CONCENTRATIONS AND REMOVAL RATES

A Dissertation

by

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ABSTRACT

Polycyclic Aromatic Hydrocarbons (PAHs) are toxic constituents found in crude oils, which can be removed from the water column through a combination of processes: evaporation, sedimentation, photo-oxidation and/or biodegradation, collectively termed weathering. Marine snow consists of many particles including bacteria, phytoplankton, mineral particles, fecal pellets and aggregates and plays an important role in the process of removing PAHs from the water column through sedimentation and enhanced biodegradation. The microbial community produces exopolymeric substances (EPS) in response to stresses including exposure to petroleum may lead to excess production of marine snow, therefore affecting the biodegredation and transport and fate of PAHs. This research hypothesizes that the PAH removal from the water column is enhanced by microbial activity in the presence of petroleum and petroleum plus Corexit.

In this study, mesocosm experiments were used to investigate PAH half-lives when petroleum and dispersants are present. The first four mesocosm experiments were undertaken with water collected from near shore or off-shore locations in the Gulf of Mexico. As part of these studies, oil and oil plus dispersant mixtures known as WAF (water accommodated oil fraction) and CEWAF (chemically enhanced water accommodated fraction) were generated

in 130 L baffled recirculation tanks, and ~80 L transferred to the mesocosms. A 1:10 dilution of the CEWAF (DCEWAF) was an additional mesocosm treatment. Control treatments with no oil or dispersant were used for comparison. Concentrated phytoplankton collected from Galveston Bay were added to all mesocosm tanks. In mesocosm 3 (M3) and 4 (M4) f/20 nutrient additions were made. Total scanning fluorescence (TSF) analysis was performed to determine estimate oil equivalents (EOE) concentrations at the start, during and at the end of the experiment. PAH composition and concentration were determined using Gas Chromatography/Mass Spectroscopy (GC/MS). The concentrations of EOE and PAH as well as changes in the PAH composition of the WAF, DCEWAF and CEWAF over time were determined. Biomarker data were measured in selected samples in order to investigate the biodegradation process.

The mesocosm experiments were designed to: 1) simulate the conditions of DWH oil spill using WAF, DCEWAF and CEWAF generated from a baffled recirculating system; 2) establish a relationship between EOE measured by TSF that allows for real time oil concentration estimates in mesocosm experiments; 3) compare PAH removal pattern under different biological conditions and 4) examine the impact of Corexit addition in the removal half-lives of PAH, providing additional information for evaluation of future usage of Corexit during marine oil spills.

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NOMENCLATURE

ADDOMEx	Aggregation and Degradation of Dispersants and Oil by Microbial Exopolymers
AF	Amplification Factor
BRT	Baffled Recirculation Tanks
CEWAF	Chemically Enhanced Water Accommodated oil Fraction
CROSERF	Chemical Response to Oil Spills Ecological Effects Research Forum
DBTs	Dibenzothiophenes
DCEWAF	Diluted Chemically Enhanced Water Accommodated oil Fraction
DCM	Dichloromethane
DWH	Deepwater Horizon
EOE	Estimated Oil Equivalent
EPS	Exopolymeric Substances
GC/FID	Gas Chromatography/Flame Ionization Detector
GC-MS	Gas Chromatography coupled with Mass Spectrometry
GERG	Geochemical and Environmental Research Group
HMW	High Molecular Weight hydrocarbons
LMW	Low Molecular Weight hydrocarbons
M1	Mesocosm 1
M2	Mesocosm 2

M3	Mesocosm 3
M4	Mesocosm 4
M5	Mesocosm 5
MOS	Marine Oil Snow
NOAA	National Oceanic and Atmospheric Administration
PAH	Polycyclic Aromatic Hydrocarbons
PHENs	Phenanthrenes
ppm	parts per million
rpm	revolutions per minute
UCM	Unresolved Complex Mixtures
WAF	Water Accommodated oil Fraction
TPH	total petroleum hydrocarbons
TR	total resolved peaks

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INTRODUCTION

On April 20, 2010, in the northern Gulf of Mexico, the deep-sea petroleum-drilling rig *Deepwater Horizon* (DWH) owned by *British Petroleum* (BP) exploded and began leaking oil (Schrope, 2010). The leak lasted for 87 days. A large amount of oil was released into the Gulf of Mexico, estimated as 3.19 million barrels by a court decree (Wade et al., 2016a). Oil and natural gas were ejected at a rapid rate, leading to dispersion of oil into droplets and resulting in surface oil slicks as well as the formation of deep-water plumes enriched in oil, dissolved gas, and gas hydrates at depths between 900 and 1200 m (Joye, 2015). The majority (>65%) of the oil was estimated to have risen to the sea surface after the DWH oil spill due to its lower density compared to water (Liu et al., 2016). It is estimated that ~25% of the spilled oil was collected or removed from the environment using immediate response methods such as pumping, skimming, and burning. The remaining 75% remained in the environment (Kerr, 2010). As a part of the oil spill response, ~1.8 million gallons of COREXIT dispersant (mainly COREXIT 9500, but also COREXIT 9527 formulation) were sprayed on the surface of the ocean and directly into the leaking oil at the wellhead at a depth of 1500 m (Bælum et al., 2012). This was reported to be the first large-scale applications of dispersants at depth (Kujawinski et al., 2011). Therefore, little is known about how the hazardous

polycyclic aromatic hydrocarbons (PAHs) component in the spill oil will be affected by the direct application of dispersants at depth.

PAHs are one of the principal contaminant classes of concern in oil spills because they are toxic and/or carcinogenic to humans and wildlife (Allan et al., 2012). Petroleum hydrocarbons are usually removed rapidly from surface waters, due to weathering processes including evaporation, dissolution, biodegradation, and photooxidation (Fingas, 1999, Liu et al., 2012, Guitart et al., 2010). However, some of the PAHs are reported to be persistent components after oil spills, probably due to their slow degradation rates (Peacock et al., 2007). A number of laboratory experiments have been carried out to study the degradation process of PAHs in seawater (Gearing et al., 1980, Whitney, 1984, Yamada et al., 2003, Zhou et al., 2013, Morales-McDevitt, 2017). However, the fate, transport, and transformation of PAHs and their degradation pathways and mechanisms in the water column remain poorly understood.

Biodegradation has been long considered to be one of the most important processes that remove spilled oil from the water column (Atlas and Hazen, 2011, Wang et al., 2016). Changes in microbial community structure were observed in the aftermath of the DWH oil spill, including a large bloom of hydrocarbon-degrading Gammaproteobacteria (Hazen et al., 2010). Alkane-

degrading bacteria from the order Oceanospirillales and obligate PAH degraders of the genus Cycloclasticus were observed to flourish during the bloom (Hazen et al., 2010, Valentine et al., 2010, Bælum et al., 2012). However, relatively little is known about how microbial communities respond to oil and dispersants at the molecular and chemical levels (Quigg et al., 2016), while the application of dispersants may further complicate the situation.

Marine Oil Snow (MOS) is formed in the presence of oil and may play an important role in sedimentation and biodegradation of hydrocarbons (Arnosti et al., 2016). Natural marine snow in direct association with a visible oil layer was observed floating on the surface of the impacted region shortly after the DWH oil spill (Passow et al., 2012). The exopolymeric substances (EPS) are thought to be produced by phytoplankton and bacteria as a microbial response to environmental stresses, in this case oil (Passow et al., 2012, Passow, 2016). Less than a month later, no marine snow was observed in the surface water at the same location (Passow et al., 2012). Therefore, MOS may be an important contributor to the sedimentation and degradation of oil during a spill.

It is imperative to understand the processes and pathways involved with MOS formation and if its formation aids in oil sedimentation and biodegradation. Therefore, the Gulf of Mexico Research Initiative (GOMRI) funded the Aggregation and Degradation of Oil and Dispersants by Microbial Exopolymers

(ADDOMEx) consortium to perform mesocosm studies to provide a better understanding on the role of EPS in the sedimentation and degradation of oil.

The objective of the ADDOMEx consortium is to establish a mechanistic understanding for the interactions of oil and oil plus dispersant with EPS under various environmental conditions. The main hypothesis is that bacteria and phytoplankton respond to oil and oil plus COREXIT 9500 by producing EPS, which interact with minerals, organic particles and organisms, and consequently influence the fate, distribution and potential effects of these hydrocarbon pollutants. In addition, it is proposed that in the presence of oil and oil plus COREXIT, some members of the microbial community may use hydrocarbons as a source of carbon and energy. My research hypothesis is that Corexit addition to the water column increased the bioavailability and therefore the removal rates of PAHs. In addition, I hypothesize that oil as well as oil plus Corexit, lead to EPS production and increased biodegradation of oil, consequently altering the transport and fate of PAHs by encouraging sedimentation as a result of increased MOS production.

LITERATURE REVIEW

PAHs

Polycyclic Aromatic Hydrocarbons, or PAHs, are aromatic hydrocarbons with two or more fused benzene rings. PAHs are common constituents found in crude oil (Overton et al., 2016). PAHs in the environment have natural as well as anthropogenic sources. Natural sources are forest and rangeland fires, oil seeps, volcanic eruptions and exudates from trees. Anthropogenic sources of PAH include burning of fossil fuel, coal tar, wood, garbage, refuse, used lubricating oil and oil filters, municipal solid waste incineration and petroleum spills and discharge (Kaushik and Haritash, 2006). The structures of some common PAHs are shown in Figure 1:

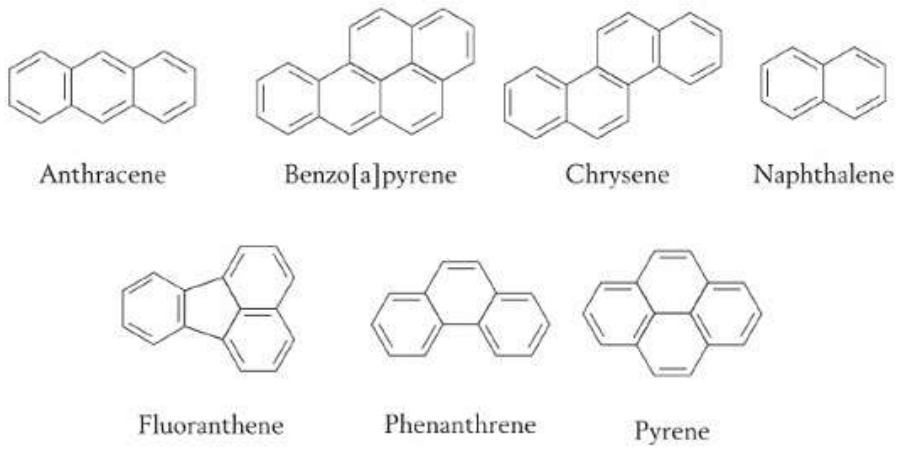


Figure 1. Examples of some common PAHs

As members of the Persistent Organic Pollutants (POPs) family (Wania and Mackay, 1996), PAHs are well known as carcinogens, mutagens, and

teratogens and therefore pose a serious threat to the health of humans and wildlife (Boström et al., 2002). PAHs are highly mobile in the environment: they are able to distribute across air, soil, and water bodies where their presence is ubiquitous (Sverdrup et al., 2002). PAHs can even be found in water samples collected off the remote area of the Greenland coast (Andelman and Suess, 1970). A low-level PAH background can be detected in the Gulf of Mexico due to deepwater oil seepage and riverine inputs; background levels of PAH are higher on the continental shelf, where PAHs may be brought in from river outlets carrying sediment from terrigenous sources (Rosenheim et al., 2016). Relatively few data on the concentration of PAHs in seawater are available, though they may produce direct toxic effects on organisms and transport with surface wave and current (McGrath and Di Toro, 2009).The water solubility and volatility of PAHs decreases as their molecular weight increases; however, low water concentrations of PAHs can be environmentally relevant due to their potential to bioaccumulate in organisms (Carls and Thedinga, 2010).

PAHs can be removed from the water column through any of the following processes: evaporation, sedimentation, photooxidation and/or biodegradation. Certain diagnostic ratios of PAH compounds may be used to track the weathering process in petroleum (Douglas et al., 1996, Olson et al., 2017). Evaporation is the most important and rapid process in the weathering of petroleum hydrocarbons for surface spills (McAuliffe, 1989). It can account

for about 75% volume loss for light crude oils and 40% for medium crudes within a few days of a surface oil spill incident (National Research Council, 2003). Naphthalene and alkyl-naphthalenes may evaporate within 24 hours within a spill (Stout and Wang, 2007). One thing to note is that the existence of dispersants may reduce the rate at which certain oil components evaporate (Gong et al., 2015). Low Molecular Weight (LMW) PAHs are also prone to biodegradation while High Molecular Weight (HMW) PAHs with more than 4 benzene rings are less likely to biodegrade in the aqueous phase and are often adsorbed onto particles and removed to the sediments (Lee et al., 1978, Hinga et al., 1986). HMW PAHs in the environment overall are much more persistent than LMW ones (Yamada et al., 2003). Photooxidation is an important pathway to remove PAHs, especially HMW ones from an aquatic system. Photooxidation in the aqueous phase may occur within several minutes after PAHs are introduced into the system (Hinga et al., 1986). PAHs are more sensitive to photooxidation than aliphatic hydrocarbons in oils, and alkyl substitution can further increase the sensitivity of parent PAHs (National Research Council, 2005). The photo-sensitivity of PAHs also increases with the number of conjugated aromatic rings (Kochany and Maguire, 1994, Liu et al., 2016). However, aromatic thiophene compounds such as dibenzothiophene are more resistant to photo-oxidization compared to the aliphatic sulfur compounds (National Research Council, 2005). Studies have shown that oil, especially dispersed oil, has greater toxicity when exposed to natural sunlight (Barron et

al., 2003, Kirby et al., 2007). This may result from the polar oxygenated species generated from photooxidation process being more toxic than the original PAHs (Liu and Kujawinski, 2015).

A common approach to determine PAH concentrations in environmental samples is gas chromatography coupled with mass spectrometry (GC-MS) (Wang and Fingas, 1995, Poster et al., 2006). Methods such as gas chromatography-flame ion detection (GC-FID) and high-performance liquid chromatography (HPLC) are also viable techniques for the purpose of PAH quantification. These chromatography techniques are sensitive, however they require extraction and concentration procedures prior to analysis. In addition, a great deal of time is spent on the quantitative measurement and interpretation of the data (Christensen et al., 2005, Bugden et al., 2008). Fluorescence spectroscopy has provided another working solution for the real-time detection of PAHs (Apicella et al., 2004). It allows more direct and rapid interpretive analysis for aquatic samples and is a useful addition to chromatography techniques.

It was noticed in previous studies that PAHs may also migrate vertically along with other petroleum hydrocarbons (Lee and Anderson, 1977, Lee et al., 1978), and certain components may persist for a long time (Gearing et al., 1980). Measurements of PAH concentrations in the water column after the

DWH oil incident indicate water concentrations decreased rapidly after the discharge of oil stopped (Wade et al., 2016a). PAHs are preferentially adsorbed onto particulate matter; therefore, bottom sediments may act as a reservoir for these hydrophobic pollutants in aquatic environment (Budzinski et al., 1997). Re-suspension of the sediments may lead to increased PAH content in the surrounding water, which may have effects on aquatic organisms (Menon and Menon, 1999). Therefore, vertical transport of PAHs is a key process to be evaluated in order to determine the long-term fate and effects of oil pollution on benthic and aquatic ecosystems. It was estimated that at least 2%-15% of the spilled oil in Deepwater Horizon eventually reached the seafloor through sedimentation (Passow and Ziervogel, 2016).

Marine snow

Marine snow is a continuous shower of mostly organic detritus falling from the upper layers of the water column, which occurs throughout the world's oceans. Marine snow is defined as particles over 0.5 mm that consist of organic and inorganic particles including bacteria, phytoplankton, small fecal pellets and microaggregates (Alldredge and Silver, 1988). The settling of marine snow is considered one of the most important processes that delivers organic materials to the deep water and ocean floor (Alldredge and Silver, 1988).

Oil, especially dispersed oil, readily undergoes adsorption to marine snow particles (Gong et al., 2014a), which could also act as a local ‘hot spot’ for oil degradation, allowing conversion of petroleum carbon into biomass (Arnosti et al., 2016). Therefore, marine snow may play an important role in transporting oil components from the water column to the sediments (Fu et al., 2014). White et al. (2012) found some coral colonies near the wellhead suffered from widespread signs of stress and disease, including excessive mucous production, tissue loss and retracted polyps. They also discovered a brown flocculent material coating many of the affected corals, and this material possessed a fingerprint which was unique to the Macondo oil. Considering the deposition of DWH oil on corals, it was estimated that ultimately 4–31% of the DWH oil was brought down by the formation of Marine Oil Snow, or MOS (Quigg et al., 2016), although it is not entirely.clear why the number is significantly different with the number from Passow and Ziervogel (2016).

The process of marine snow formation after the DWH oil spill is still not well understood. There is a hypothesis that it was formed from mucus webs produced by oil-degrading bacteria, which exude exopolymeric substances (EPS) to allow emulsification and thus easier degradation of the oil (Passow et al., 2012). These EPS are the underlying matrix of marine snow particles (Meinhard et al., 2002) and appear to be extremely surface-active (Zhou et al., 2003). It was found recently that the rate of formation of micron-scale

aggregates of microbial cells, which are the precursors to marine snow, was directly related to the concentration of oil within the water column (Doyle et al., 2018). Therefore, it seems that not only is EPS itself of great ecological importance, but also it may play a key role in affecting the fate and transport of oil in the aftermath of a spill.

Microorganisms, including bacteria, archaea and phytoplankton, are known to be capable of biodegrading oil. The oil-degrading microorganisms are ubiquitously found in marine waters, but typically only make up a small fraction of the pre-spill communities (Bælum et al., 2012, Valentine et al., 2014). The release of petroleum hydrocarbons, including PAHs, into the water column triggers a series of complicated microbial responses (Bælum et al., 2012, Joye, 2015). For example, changes of microbial community structure were observed in the aftermath of the DWH oil spill, including a large bloom of hydrocarbon-degrading Gammaproteobacteria (Hazen et al., 2010). Alkane-degrading bacteria from the order Oceanospirillales and obligate PAH degraders of the genus Cycloclasticus were observed to flourish during the bloom (Hazen et al., 2010, Valentine et al., 2010, Bælum et al., 2012). EPS are reported to form the matrix for microbial aggregates in these hydrocarbon degrading communities and are functionally comparable to biofilms, which will allow microbes to target appropriate substrates such as oil leading to the biodegradation of oil components (Hazen et al., 2010, Valentine et al., 2010, Bælum et al., 2012).

The marine snow produced through this process is extremely sticky and allows free suspended particulates to adhere to it, leading to the formation of a rich substrate that can be continuously colonized by bacteria, increasing in biomass and dimensions over time (Passow et al., 2012, Ziervogel et al., 2012). However, despite the significant efforts made by researchers, these proposed theories remained hypotheses and much of the relationship between microbes and formation of MOS remains uncertain.

Dispersants

Dispersants were widely applied during the DWH oil spill and previous oil spill incidents. The prime motivation of using dispersants in an oil spill incident is to reduce the amount of oil reaching the shoreline (National Research Council, 2005). The main active ingredients in oil dispersants are surfactants (surface active agents). For example, Corexit 9527 consists of a mixture of both nonionic (ethoxylated mono- and trioleates) and anionic (sodium dioctyl sulfosuccinate) surfactants in an aqueous solution of ethylene glycol monobutyl ether (Scelfo and Tjeerdema, 1991). Corexit 9500 contains the same surfactants as Corexit 9527 except that it does not contain 2-butoxy ethanol (George-Ares and Clark, 2000). Both Corexit 9500 and Corexit 9527 were applied during DWH oil spill (Kujawinski et al., 2011). The dispersants work by lowering the interfacial tension between oil and water and thereby reducing the size of oil droplets and possibly allowing for a more rapid biodegradation process (Kujawinski et al.,

2011). When used under the right circumstances, the application of chemical dispersants may promote biodegradation of oil and significantly reduce the impacts of oil on sensitive shorelines and habitats (Lessard and DeMarco, 2000).

About 2 million gallons of chemical dispersants were applied to the Gulf waters to accelerate the dispersal and dissolution of PAHs following the DWH oil spill incident (Kujawinski et al., 2011). One adverse effect of dispersant usage may be increased water column organisms' exposure to crude oil (Ramseur, 2010, Ramachandran et al., 2004). Dispersant usage is also shown to significantly increase the PAH concentration (typically by 5-10 times) in the water column (Yamada et al., 2003, Couillard et al., 2005). There are reports that chemically dispersed oil may be more toxic then the original physically-dispersed oil (Cohen et al., 2003, Khan and Payne, 2005, Shafir et al., 2007), and also that dispersants may be toxic (Barron and Ka'aihue, 2003). Dispersant and dispersed oil can be transported through advective processes like surface water wave action and underwater currents; they are also able to move vertically via formation and settling of marine snow (Gong et al., 2014a). It was also demonstrated that Corexit 9500 can lead to increased sediment uptake of dispersed oil and PAHs (Gong et al., 2014b), allowing PAHs to penetrate deeper into anoxic sediments (Zuijdgeest and Huettel, 2012).

It has been shown that dispersants can reduce the evaporation of pyrene while increasing its photodegradation efficiency (Gong et al., 2015). Whether dispersants can actually improve the efficiency of the biodegradation process of oil is still being debated (Kleindienst et al., 2015). Observations of acceleration (Bælum et al., 2012, Morales-McDevitt, 2017), no or little effect (Foght and Westlake, 1982, Macías-Zamora et al., 2014) or even inhibition (Bruheim et al., 1999) have been reported. Even dispersants themselves in certain occasions may be degraded preferentially over oils (Kleindienst et al., 2015, Lindstrom and Braddock, 2002). The above studies mainly focused on the effect of biodegradation on crude oils; there is little historical evidence on biodegradation of PAHs promoted by addition of dispersants (Fingas and Banta, 2009).

The effect of dispersants on the rate of oil degradation may vary with the different chemical composition of dispersants. The class of compounds whose degradation was affected and the degree of effect varied among dispersants, even though the oil and the microbial culture were the same (Foght and Westlake, 1987). Another earlier study reported that the biodegradation of normal and branched alkanes was inhibited by Corexit 9527, while biodegradation of PAHs was not affected (Foght and Westlake, 1982).

The overall effects of Corexit 9500A on sedimentation of oil-rich marine snow is currently difficult to assess and not well understood. Very low concentrations of Corexit seem to inhibit marine snow formation in experiments and it is likely that the presence of Corexit reduced microbial marine snow formation and sedimentation after the spill (Passow, 2016). On the contrary, there is also a report that the addition of oil and dispersant greatly enhanced the bacterial growth and EPS production, resulting in increased flocculation and formation of marine snow (Fu et al., 2014). Whatever effect Corexit may have on the formation of aggregates, it needs to be considered when evaluating the advantages and disadvantages of use of dispersants including Corexit.

QUESTIONS AND ASSOCIATED HYPOTHESIS

The objective of the ADDOMEx consortium is to establish a mechanistic understanding of the interactions of oil and oil plus dispersant with EPS under various environmental conditions. The main hypothesis is that bacteria and phytoplankton respond to oil and oil plus COREXIT 9500 by producing EPS, which interact with minerals, organic particles and organisms, and consequently influence the fate, distribution and potential effects of these hydrocarbon pollutants. In addition, a second hypothesis is that in the presence of oil and oil plus Corexit, some members of the microbial community may use hydrocarbons as a source of carbon and energy. It was observed that the microbial community structure was changed after the introduction of oil and/or dispersants (Doyle et al., 2018). It was also shown that petroleum hydrocarbon may be incorporated into aggregates formed by microbes, leading to oil sedimented along with marine snow, and Corexit may enhance that process (Passow et al., 2017). These works done by the consortium mainly focused on the biological side of the mesocosms, while my research looked into the chemical aspect, which may provide supporting evidence to their hypotheses.

My research hypothesis is that Corexit addition to the water column increases the bioavailability and therefore the removal rates of PAHs. In addition, I hypothesize that oil as well as oil plus Corexit, lead to EPS production

and increased biodegradation of oil, consequently altering the transport and fate of PAHs by encouraging sedimentation as a result of increased MOS production. In order to test these hypotheses the following questions will be addressed in my research:

1. What are the initial PAH concentrations in mesocosms?
2. How does PAHs concentration in WAF, DCEWAF and CEWAF tanks change differently over time?
3. What is the cause of PAH removal? Which factor is more importantant in PAH removal, biodegradation or sedimentation?

METHODS

The ADDOMEx consortium set up a series of experiments that will potentially explain how the production of EPS by specific phytoplankton and bacteria in the presence of hydrocarbons will simultaneously protect these organisms and contribute to the degradation of oil. The research proposed for this project focuses on quantifying the estimated oil equivalents (EOE) and polycyclic aromatic hydrocarbons (PAHs) under four groups of different treatments: WAF (oil only), CEWAF (concentrated oil plus COREXIT 9500), DCEWAF (diluted oil plus COREXIT 9500), and a seawater-only control. The method section below is based on the description in publications by Morales-McDevitt (2017) and Wade et al. (2017).

The EOE were measured after the addition of a microbial or nutrient concentrate, and then periodically but at least every 24 hours for a total period of 48 to 96 hours depending on the length of the experiment. In the case of the PAH quantification, 1-4L of water (depending on the experiment) from each treatment were collected at the beginning, during and at the end of each experiment. M1 had single treatments, while M2, M3, and M4 had three replicate treatments.

Baffled recirculation system

Most studies use the CROSERF method to prepare WAF (Singer et al., 2000). In this study, an improved procedure was applied, involving the use of baffled recirculating tanks (BRT) to produce large volumes of WAF, DCEWAF and CEWAF for dosing of medium scale mesocosms (~110 L). The design of the baffled recirculation tanks is described in Wade et al. (2017). These tanks with the size of 40x40x72 cm can contain 112 L of seawater. The materials used were non-tempered glass (1/2 in thick) and transparent silicone. Four baffles with two different heights were installed in order to guide the flow of the accommodated fractions of oil and dispersant through the tank.

A Masterflex PTFE-Diaphragm Pump with Teflon heads and tubing was used to recirculate the water. The tubing was connected to two steel pumps for better stability in the system. The inflow was placed in the first chamber (left to right), and the outflow in the last (Figure 2). In addition to the diaphragm pump, mixing was aided with one Thermo Scientific magnetic stirrer and one Arrow 1750 electric stirrer.

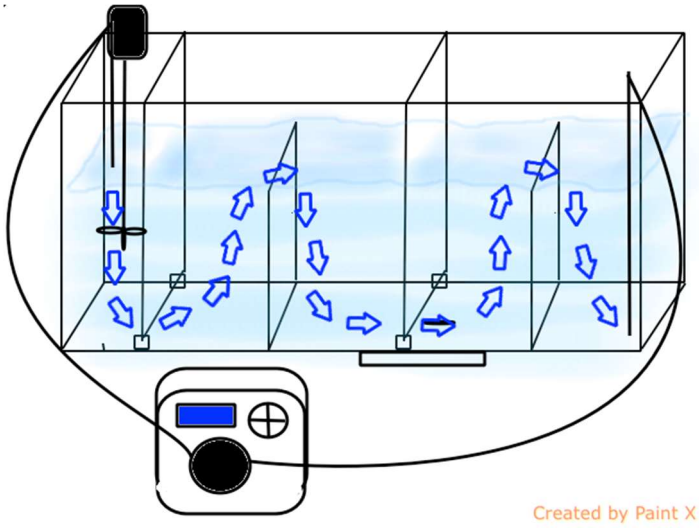


Figure 2. Scheme for baffled recirculation tanks. Picture courtesy of Morales-McDevitt (2017)

WAF and CEWAF generation

The objective of this part of the experiment was to generate reproducible amounts of WAF, CEWAF and DCEWAF at a specific concentration that were later transferred into the mesocosm tanks. The Chemical Response to Oil Spills Ecological Effects Research Forum (CROSERF) has defined water-accommodated fraction (WAF) as "*a laboratory-prepared medium derived from low energy (no vortex) mixing of oil, which is essentially free of particles of bulk material.*" (Singer et al., 2000) The CROSERF method is suitable to prepare small amount of WAF up to several liters. As hundreds of liters of water were involved in our mesocosm experiment, BRT was used as a working alternative to the CROSERF method for the WAF preparation. The oil used in this project was the Macondo surrogate oil from the Marlin Platform Dorado, which has a specific gravity of 0.86 and similar

chemical composition to the Louisiana Sweet Crude Oil spilled during the BP incident in 2010. The dispersant added to corresponding treatments was COREXIT 9500A.

Seawater (~120 L) was transferred to each baffled recirculation tank where the WAF and CEWAF were produced. The baffled recirculation tanks physically dispersed Macondo surrogate oil and dispersant (COREXIT 9500) with flow generated by the PTFE-Diaphragm pump that recirculated the seawater at 250 rpm (or 333 ml min^{-1}). In addition, an electromagnetic stirring plate and an Arrow 1750 electric stirrer, at rates no higher than 200 rpm to avoid creating a vortex in the water, were used as mixing energy sources. By using low energy mixing, dispersion and emulsification of the oil was prevented (Singer et al., 2000).

WAF subsurface concentrations in the laboratory can range from 1 to 20 ppm (Knap et al., 1983). Therefore, our experimental concentrations were expected to range from ~20 ppm (20 mg/L) in the WAF and DCEWAF. With the specific gravity of 0.86 for the Macondo surrogate oil, 24 mg/L of the oil were added to the WAF recirculation tank. In the case of the CEWAF recirculation tank, a 1:20 (1 ml dispersant plus 20 ml oil) dilution previously mixed was added to its corresponding recirculation tank. The oil is added in excess of the amount of oil required for WAF in the 130L baffled recirculation tank.

The oil content of water (or water accommodated fraction of oil) was be measured every 24 hours. The estimated oil equivalent (EOE) was measured with a Horiba Scientific Aqualog fluorometer, with the excitation wavelength of 260 nm and emission wavelength of 372 nm. After 48 hours it was assumed that the oil concentration in the water had reached its maximum, and therefore, the generation process had been completed.

Mesocosm experiments – M1 & M2

Before each experiment, a calibration curve was generated using a Macondo surrogate oil as the standard at five different concentrations and ran through the Horiba Scientific Aqualog fluorometer. The maximum intensity of its fluorescence was used to determine the calibration equation and calculate the concentrations of the samples to be analyzed. The coefficients of determination, or R^2, were above 0.996 for all the calibration curves produced.

The process of generating WAF and CEWAF was described in Wade et al. (2017). During the last week of July and third week of October of 2015, the NOAA (National Oceanic and Atmospheric Administration) vessel, the “Manta”, collected for the ADDOMEx consortium 1000 L of seawater and 4 L of filtered microorganisms near Galveston Bay. The seawater (34 psu) was processed through a charcoal filter to remove large particulates and debris. Plankton (≥63

μm) were collected from the TAMUG dock using a net and transferred into polycarbonate bottles. For the Mesocosm 1 experiment, 4 mesocosm tanks were treated in the following way. The control tank was filled with the seawater directly from the storage tank. This seawater was also used to fill each of the baffled recirculating tanks for WAF and CEWAF production. The WAF was prepared by mixing a total of 24 ml (2 ml to start, 2 mL after 1 hr, then 5 ml at ~ 2, 3, 4 and 5 hrs) of Macondo Surrogate oil into the seawater. Total mixing time from the start of oil addition to transfer to the mesocosms was 18 hrs. The WAF (79 L) was transferred to a tank and homogenized. CEWAF production involved mixing Corexit 9500 with Macondo Surrogate oil in a ratio of 1:20 (Corexit to oil, V/V) and 24 ml of this mixture (2 ml to start, 2 ml after 1 hr, then 5 ml at ~ 2, 3, 4 and 5 hrs total of 24 ml) was added to 130 L of seawater and mixed for 18 hrs. The CEWAF (79 L) was transferred to the corresponding mesocosm tanks and homogenized. The dilute CEWAF (DCEWAF) mesocosm treatment was produced by adding 9 L of CEWAF to 70 L of the original seawater for a total volume of 79 L. The plankton collected earlier was added to the mesocosms.

For the Mesocosm 2 experiment, WAF was prepared by mixing 25 ml (5 ml ~ every 30 min for 2.5 hrs) of Macondo Surrogate oil into 130 L of seawater then mixing for 12 to 24 hrs. The WAF was then introduced into the WAF mesocosm tanks and filled to 87 L and homogenized. For the CEWAF, Corexit was mixed with oil at a ratio of 1:20 and 25 ml of this mixture (5 ml every 30 min

for 2.5 hrs) of Macondo Surrogate oil plus Corexit were added to 130 L of seawater, which was mixed for 8 to 24 hrs prior to being transferred to the mesocosm tanks. The CEWAF was then introduced into the CEWAF mesocosm tanks and filled to 87 L and mixed. Diluted CEWAF (DCEWAF) was prepared by mixing 9 L of CEWAF with 78 L of the original seawater for a total volume of 87 L.

During the first experiment setup, four 90 L mesocosm tanks were filled with 79 L of its corresponding accommodated oil (WAF) or oil plus dispersant fraction (CEWAF and DCEWAF). The first tank used as the control was filled with untreated prefiltered seawater, the second tank with WAF, the third with DCEWAF, and the last one with CEWAF. For the second, third, and fourth experiments each treatment was done in triplicate, having a total of 12 mesocosm tanks. For the WAF, CEWAF and DCEWAF controls, a liter of non-enriched water was taken from each treatment and kept in amber bottles.

Every 24 h 5 ml of water were taken out of the triplicates of each treatment, which were then extracted with 5ml of dichloromethane (DCM). The DCM fraction of each experiment was transferred into cuvettes and analyzed in the Horiba fluorometer. In order to accurately determine the EOE, all samples were compared to the calibration curve.

Mesocosm experiments – M3 & M4

For the Mesocosm 3 experiment, twelve 100L mesocosm tanks were filled with Gulf of Mexico seawater collected from the Flower Gardens National Marine Sanctuary Area (27° 53.4180'N; 94° 2.2020'W) which is located ~120 miles off the coast of Galveston (TX). For the Mesocosm 4, the seawater was collected from the Texas coastline, near the Texas Automated Buoy System (TABS) buoy R (29° 38.1000’N, 93° 38.5020’W) which is located ~100 miles away from Galveston (TX). The sampling locations are shown in Figure 3. The other processes stayed identical for M3 and M4. Four treatments were prepared in triplicate. Control tanks were filled with seawater. WAF, CEWAF and DCEWAF treatments were prepared in the same way as in the Mesocosm 2 experiment.

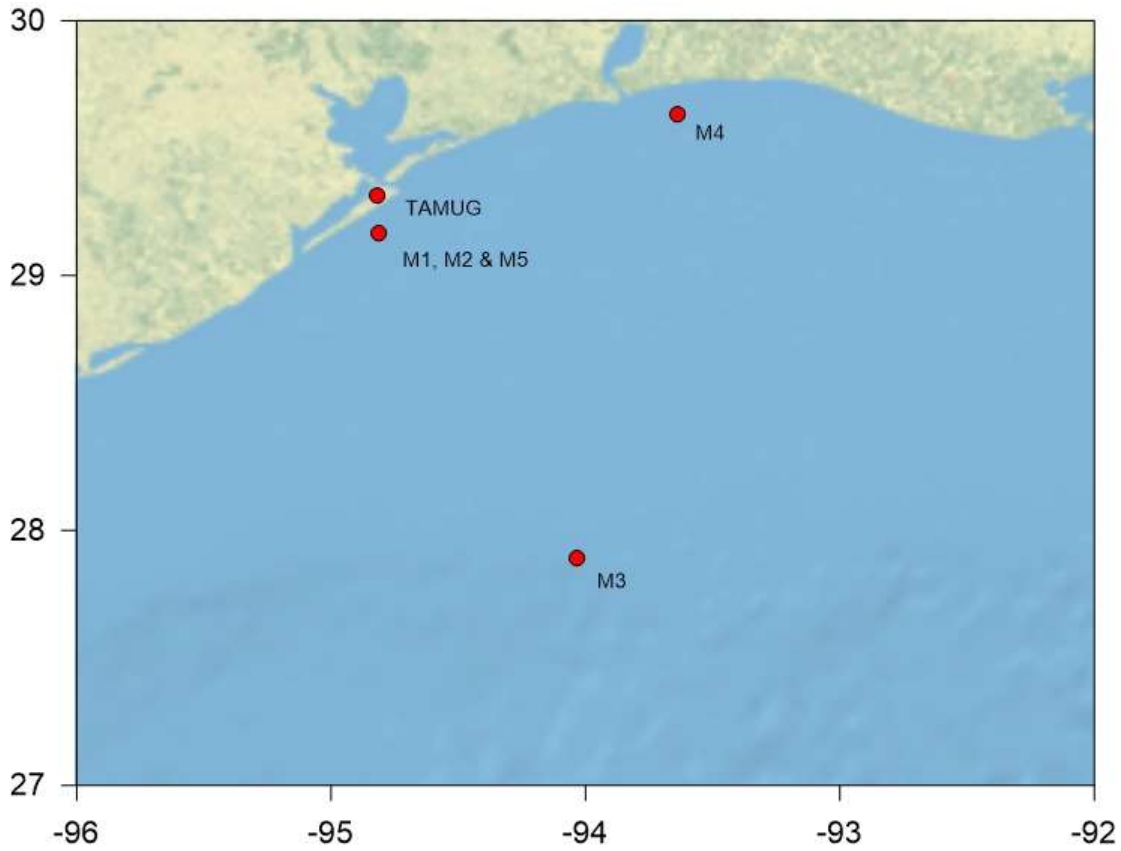


Figure 3. Map of sampling locations

A general enriched seawater medium (f/20 medium) designed for coastal marine algae was prepared according to the specifications of Guillard and Ryther (1962) and Guillard (1975), using the guidelines of the National Center for Marine Algae and Microbiota. Shortly after the treatments were transferred to the mesocosm tanks, the phytoplankton and/or 20 ml of nutrient concentrate was added to each treatment and the water was stirred. Sampling commenced and this was defined as time zero.

Mesocosm experiments – M5

The Mesocosm 5 experiment was carried out between May 23rd, 2017 and June 7th, 2017, with a total of 15 days. 18 mesocosm tanks with the volume of 100 L were filled with seawater collected from Galveston Bay. The WAF and DCEWAF was prepared in the same way as in the Mesocosm 2 experiment except that the mixing time was 4 hours instead of 24 hours in M2 experiment. The mesocosm tanks were divided into 3 groups, each containing 6 replicates of Control, WAF and DCEWAF treatments. Among the 6 replicate treatments, 3 were sacrificed and had bulk water samples collected to measure the PAH concentrations on Day 3; the other 3 lasted until the end of the experiment, which was on Day 15.

The EOE concentration in the WAF, CEWAF and DCEWAF tanks were measured at intervals of ~24 hours. Optimum wavelengths for the surrogate oil were found at 260 nm for excitation and 372 nm for emission prior to the experiment. A calibration curve was generated using the Macondo surrogate oil prepared at five concentrations ranging from 100 to 5000 ug/L.

Estimated oil equivalents (EOE)

The estimated oil equivalents (EOE) were determined using Macondo surrogate oil as the calibration standard (Wade et al., 2011b). Before each experiment, a calibration curve was generated using a Macondo surrogate

standard oil dissolved in dichloromethane (DCM) at five concentrations between 100 and 5000 ug/L and analyzed on a Horiba Scientific Aqualog fluorometer. The linear calibration curve with an R^2 of greater than 0.999 was used to calculate EOE concentrations for DCM extracts of mesocosm water samples. The EOE concentration in the WAF, CEWAF and DCEWAF were measured by fluorescence periodically. For each measurement, 5-70 ml of seawater was collected from each mesocosm and extracted with 5 ml of dichloromethane (DCM). As the experiment went on, it became necessary to collect larger volumes of seawater to compensate for decreasing EOE concentration with time. The DCM fraction was transferred into cuvettes and analyzed for EOE by Total Scanning Fluorescence (TSF).

Hydrocarbons analysis

For the Mesocosm 2 experiment, ~4 L of seawater were set aside at time zero and at the end of each experiment and preserved with DCM. For the Mesocosm 3 and 4, ~1L of seawater samples were collected every 24 hours beginning at time zero. The end point was at 96 hours (4 days) after T0 for Mesocosm 3 and 72 hours (3 days) after T0 for Mesocosm 4. For the Mesocosm 5, ~3.5 L of seawater samples were collected on the beginning, Day 3 and Day 15 respectively. Samples were later transported to the GERG facilities for analysis. Prior to the extraction, 100 μL of PAH recovery standard solution (containing 5 deuterated PAHs: d8-naphthalene, d10-acenaphthene,

d10-phenanthrene, d10-chrysene and d12-perylene) was added to each sample to determine the recovery rate. For M5 samples, 100 μL of aliphatic recovery standard solution containing deuterated n-C12, n-C20, n-C24 and n-C30 was added as well. These samples were extracted with dichloromethane (DCM) two times, with volumes of 100 ml and 50 ml respectively. The DCM fractions were combined, concentrated and further purified with alumina/silica gel (Al/Si, 10g/20g) chromatographic columns (300 × 13 mm i.d.). The hydrocarbons in the sample were eluted from the column using 200 ml of DCM/pentane mixture (1/1, v/v). The eluted faction was then concentrated to a final volume of 1 mL and spiked with PAH recovery standards (containing d10-fluorene and d12-benzo[a]pyrene) for GC/MS analysis.

Aliphatic hydrocarbons and total petroleum hydrocarbon (TPH) analysis were carried out for M5 samples. An Agilent 7890 gas chromatograph with a flame ionization detector (GC/FID) (Wade et al., 2011b) with an Agilent DB-5MS fused capillary column (30 m long x 0.25 mm I.D. with a 0.25μ film thickness) was utilized for this analysis. The oven program was set at 60°C for 1 min, then 6°C/min to 300˚C and held for 10 min. The n-alkanes ranging from n-C10 to n-C35, and the isoprenoids pristane and phytane were quantified using relative response factors calculated from the response of the analyte in calibration standards. Total resolved (TR), unresolved complex mixture (UCM) and total petroleum hydrocarbons (TPH) concentrations were calculated using

an average of the relative response factors for all n-alkanes and isoprenoids present in the calibration standard and the relevant areas. The UCM was composed of thousands of hydrocarbons which are not resolved as peaks from each other (co-elute), which produced a hump in the gas chromatogram. Total resolved hydrocarbons was calculated by summing the area from all peaks from the retention time of n-C10 to the retention time for n-C35 with the recovery and internal standard areas removed. TPH was the total integrated area above a straight line starting at the retention time of n-C10 to n-C35 with the recovery and internal standard areas removed by subtraction of the total integrated area from a blank to correct for any baseline rise. UCM concentration was the difference between TPH and TR (Wade et al., 2011b).

The polycyclic aromatic hydrocarbon (PAH) quantification was determined using the *"Standard Operating Procedure for Quantitative Determination of Polynuclear Aromatic Hydrocarbons by Gas Chromatography/Mass Spectrometry Using Selected Ion Monitoring Mode (SOP-9733)"*. The PAHs were analyzed with a Hewlett-Packard 6890 gas chromatograph (GC) coupled with a Hewlett-Packard 5973 mass selective detector. A laboratory reference sample (diluted oil sample) was analyzed with each batch of samples to confirm GC-MS/SIM system performance and calibration. Instrumental calibrations were checked by injection of a mid-level calibration solution. Separation of PAHs was accomplished with a DB-5 MS

fused silica capillary column (30 m×0.25 mm i.d.,0.25 μm film thickness, J&W Scientific). The oven temperature was programmed to increase from an initial temperature of 60°C to 150°C at 15°C min^{-1}, then 5°C min^{-1} to 220°C, and finally at 10°C min^{-1} to a final temperature of 300°C with a final holding time of 10 min. Petroleum biomarkers were also analyzed for selected samples from M5. Target compounds were obtained by comparing the gas chromatographic peaks of the sample with those of the standard. The PAHs were identified based on the comparison of the retention time and mass spectrum of selected ions with the calibration standards. Alkylated PAHs were quantified based on the response of the parent PAHs (e.g. naphthalene response factor was used to determine naphthalene with 1-4 substituted carbons). Target compounds were obtained by comparing the gas chromatographic peaks of the sample with those of the standard. After the peaks have met all the qualitative identification criteria, the concentrations of the target compounds were calculated using the following equation:

$$C = \frac{(A_S)(C_{SU})}{(A_{SU})(\overline{RRF})(Sa)}$$

where:

C = Concentration in sample (ng/gram or ng/liter).

Sa = Sample amount (grams, liters).

A_S = Area of the quantitation ion for the target compound to be measured.

A_{SU} = Area of the quantitation ion for the recovery standard.

C_{SU} = Amount of recovery standard added to each extract (ng).

$\overline{RRF}$ = Average response factor

It was necessary to calculate the percent recovery of the five recovery standards in the sample extract by using the following equation:

$$\% \text{ recovery} = \frac{(A_{SU} \times C_{IS})}{(C_{SU} \times A_{IS} \times \overline{RRF}_{SU})}$$

where:

A_{IS} = Area of the quantitation ion for the appropriate internal standard

A_{SU} = Area of the quantitation ion for the recovery standard

C_{SU} = ng of deuterated recovery standard added to the sample

C_{IS} = ng of deuterated internal standard added to the sample extract

$\overline{RRF}_{SU}$ = Average response factor for the recovery standard based on the internal standard from the initial calibration.

RESULTS AND DISCUSSION

Mesocosm 1 (M1) was a pilot study and the data will not be reported here. This research will focus on the data provided by M2, M3 and M4 experiments, especially M3 and M4 since they provide more detailed data on the concentration of PAHs over time.

Estimated oil equivalents (EOE)

All fluorescence signals were below detection limit for all samples collected from control treatments. Therefore, the EOE of control samples were determined at 0 mg/L. For the other treatments, the measured EOE concentrations are provided in Table 1. EOEs decrease with time in all treatments in all 3 mesocosm experiments. The decay rates, which ranges from -0.22 d^{-1} to -0.72 d^{-1}, were calculated assuming EOEs' removal follows a first-order exponential rate. EOE removal was in good agreement with the exponential model (R^2>0.90) in most treatments for the 3 mesocosm experiments.

Table 1. Estimated Oil Equivalents (EOE) values measured and their change rates in M2-M4 experiments (in mean ± standard deviation format). R^2 calculated from first-order reaction model.

	Mesocosm 2			Mesocosm 3			Mesocosm 4		
Time elapsed	WAF (mg/L)	DCEWAF (mg/L)	CEWAF (mg/L)	WAF (mg/L)	DCEWAF (mg/L)	CEWAF (mg/L)	WAF (mg/L)	DCEWAF (mg/L)	CEWAF (mg/L)
0	0.3±0.01	2.7±0.4	41.5±2.8	0.7±0.5	6.2±1.3	39.0±0.8	0.3±0.03	8.1±1.0	81.1±20.6
24	0.09±0.01	1.6±0.1	19.5±3.4	0.4±0.2	5.7±0.3	24.2±2.8	0.1±0.04	5.4±0.9	38.8±3.5
48	0.07±0.02	1.3±0.07	25. 8±3.7	0.3±0.15	4.2±0.6	19.6±2.5	0.09±0.01	4.0±1.0	33.2±4.6
72	0.06±0.01	1.0±0.07	17.3±4.9	0.1±0.1	3.2±0.8	12.4±2.0	0.03±0.01	1.8±1.1	19.8±1.3
96	-	-	-	0.05±0.04	2.7±0.2	8.2±2.6	-	-	-
Decay rate (d^{-1})	-0.46	-0.31	-0.24	-0.65	-0.22	-0.38	-0.72	-0.48	-0.43
Half-life (d)	1.49	2.23	2.96	1.05	3.13	1.83	0.96	1.46	1.58
R^2	0.83	0.9437	0.6035	0.9693	0.9767	0.9896	0.9541	0.9568	0.9443

WAF: Water Accommodated Fraction

DCEWAF: Diluted Chemically Enhanced Water Accommodated Fraction

CEWAF: Chemically Enhanced Water Accommodated Fraction

±: standard deviation

-: no data taken

The half-lives for EOE in the same treatment across 3 experiments are roughly in the order of M2>M3>M4. M3 and M4 had lower half-lives than M2, possibly due to nutrients added lowering the half-life due to increased biodegradation (Walworth and Reynolds, 1995, Coulon et al., 2005). Within the same experiment, the WAF treatment always has the lowest half-life and CEWAF has the highest half-life (except M3, where DCEWAF has the highest half-life). The average EOE concentration of CEWAF tank in M4 at time 0 was significantly higher than M2 and M3, demonstrating the variability in the process of producing CEWAF (Wade et al., 2017). This variability is inherent in the production of WAF in large volumes; similar variability has been reported by others (Gearing et al., 1980, Knap et al., 1983).

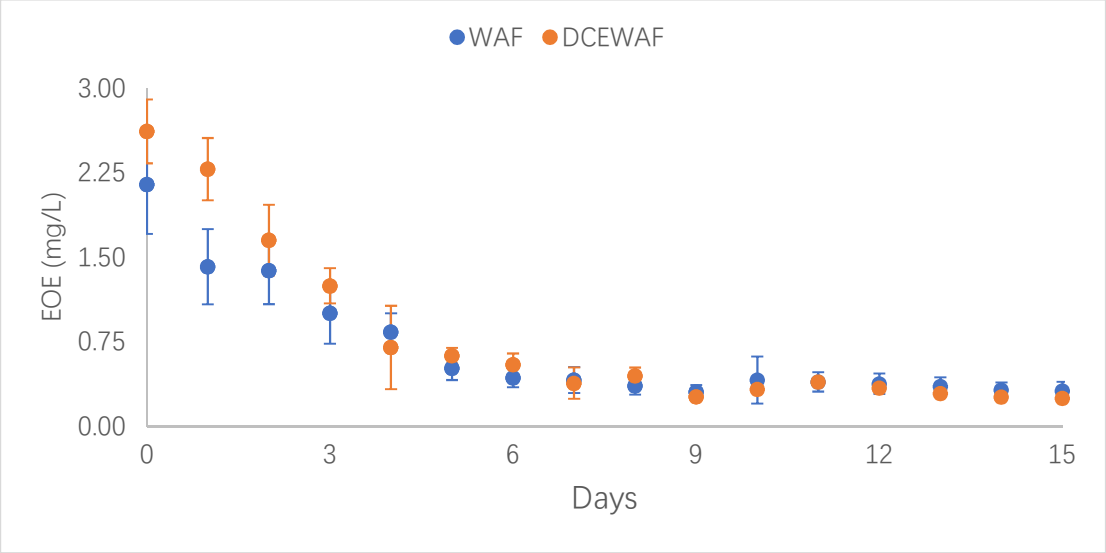


Figure 4. Mesocosom 5 EOE (mg/L) WAF and DCEWAF versus Time in days. Error bars indicate standard deviation among replicates (6 replicates before Day 3 and 3 replicates after Day 3).

Figure 4 shows the EOE for M5 experiment. The EOE concentrations with time during the experiment were measured at in replicate mesocosms at 16 time points (Day 0 to Day 15). The average initial DCEWAF EOE concentration (2.62±0.28 mg/L) was higher than the average initial WAF EOE concentration (2.15±1.44 mg/L), but with overlapping standard deviations (SD). Only on day 1 is the average DCEWAF EOE concentration higher than the WAF EOE concentration with SD not overlapping. The conclusion is that while the average EOE DCEWAF concentration was higher at the start of the experiment the concentrations of EOE in the WAF and DCEWAF were the same within the one SD for all except day 1. This shows that the presence of Corexit in treatments with similar oil concentrations had no effect on the oil concentration over 15-day time frame of this experiment.

The EOE in both the WAF and the DCEWAF had a declining trend similar to the previous studies, before it reached the minimum at Day 9. There was a small fluctuation in EOE concentration after Day 9 in both treatments. Based on the EOE concentrations at Day 0 and Day 3, the half-lives of EOE were 2.7 days for WAF and 2.8 days for DCEWAF, which is not a significant difference between the treatments. Both half-lives and initial concentrations of EOE in M5 experiment resemble the DCEWAF treatment of M2 and M3.

Aliphatic Hydrocarbons

The DWH oil and the Macondo surrogate oil which was utilized to generate WAF and CEWAF are typical light Louisiana crude oils composed of saturated n-alkanes, PAHs and alkylated PAHs (Liu et al., 2012). Aliphatic hydrocarbon analysis was carried out for samples from M5. Analytes included normal alkanes from C-10 to C-35 and two isoprenoids: pristane and phytane. Table 2 shows the result of hydrocarbon analysis for M5.

Table 2. Concentrations of Alkanes, TR (Total Resolved), TPH (Total Petroleum Hydrocarbons) and UCM (Unresolved Component Mixture) for M5. The concentrations of hydrocarbons in control treatment was too low and not shown here.

	Day	Alkanes		Total Resolved		TPH		UCM	
		Conc (μg/L)	RSD (%)	Conc (μg/L)	RSD (%)	Conc (μg/L)	RSD (%)	Conc (μg/L)	RSD (%)
WAF	0	171.26	28.7%	469.5	31.4%	1305.3	23.9%	835.8	21.7%
	3	24.01	48.0%	64.7	25.3%	411.7	28.5%	347.0	29.0%
	15	3.66	66.8%	27.8	36.6%	235.1	24.0%	207.3	22.4%
DCEWAF	0	218.80	20.2%	572.3	7.2%	1439.0	21.4%	270.2	31.2%
	3	48.48	5.0%	213.0	3.9%	506.1	39.4%	192.7	65.8%
	15	8.65	41.9%	76.8	16.8%	363.6	28.1%	101.1	35.3%

TPH is often measured in oil spill events to provide an estimate of the concentration of higher molecular weight (HMW) hydrocarbons present in the samples (Wade et al., 2016b). The TPH concentrations of control treatments varied from 52.2 to 86.0 μg/L, which is about the same level as historical background TPH data in the Gulf of Mexico (Wade et al., 2016b), and significantly lower than the TPH concentrations of WAF and DCEWAF

treatment in M5. At Day 3, about 14% of the alkanes in WAF treatment and 23% of the alkanes in DCEWAF treatment remained in the mesocosms. EOE concentrations, which are correlated with PAH concentration due to their fluorescent nature, remained similar at Day 3 for WAF (46%) and DCEWAF (48%), suggesting that aliphatic hydrocarbons are removed faster than PAHs. Upon completion of this experiment, 2.1% of the initial alkane content remained in the WAF treatment, and 4.0% for DCEWAF treatment.

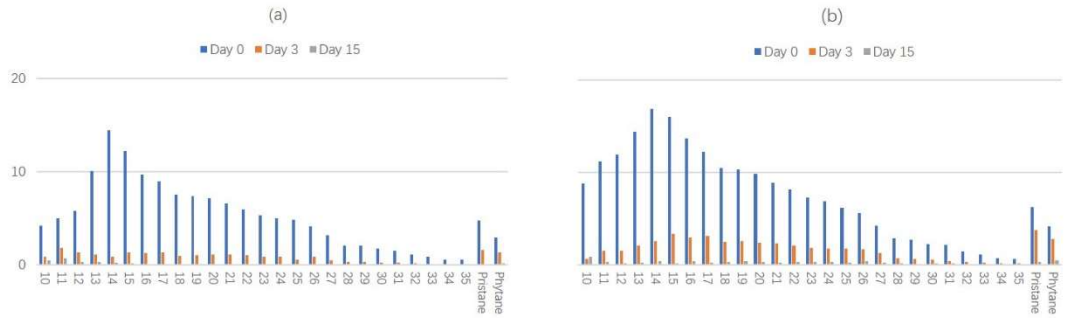


Figure 5. Concentration profiles of normal alkanes, pristane and phytane in (a) WAF and (b) DCEWAF samples of M5

Figure 5 shows the result for individual hydrocarbons. The alkane concentrations in control treatment was too low and not shown here. The concentrations of n-alkanes were all lower for WAF treatments. The n-alkanes were removed rapidly in both WAF and DCWAF treatments. At Day 3, the DCEWAF treatment had higher concentrations of n-alkanes, pristane and phytane. At Day 15, the high molecular weight portion (n-C28 - n-C35) was below the detection limit for the WAF treatment, while having low but observable concentrations in the DCEWAF treatment.

Table 3. Half-lives of normal alkanes, pristane and phytane in WAF and DCEWAF treatments

Alkanes	Half-life (WAF)	Half-life (DCEWAF)
n-C10	1.34	0.82
n-C11	2.09	1.05
n-C12	1.46	1.02
n-C13	1.00	1.03
n-C14	0.76	1.12
n-C15	0.94	1.35
n-C16	1.03	1.38
n-C17	1.10	1.54
n-C18	1.04	1.46
n-C19	1.08	1.48
n-C20	1.12	1.48
n-C21	1.18	1.57
n-C22	1.18	1.55
n-C23	1.16	1.52
n-C24	1.19	1.54
n-C25	1.01	1.69
n-C26	1.33	1.73
n-C27	1.10	1.71
n-C28	1.19	1.51
n-C29	1.10	1.53
n-C30	1.03	1.54
n-C31	1.11	1.35
n-C32	1.02	1.56
n-C33	1.06	1.43
n-C34	1.07	1.43
n-C35	0.97	1.45
Total n-alkanes	1.15	1.43
Pristane	1.90	4.06
Phytane	2.77	5.28

Table 3 shows the half-lives of aliphatic hydrocarbons measured in M5 experiment. Pristane and phytane had the longest half-lives among all alkane analytes in this experiment, due to their branched structures being more

resistant to biodegradation. The DCEWAF treatment had longer half-lives for most n-alkanes except n-C10 to n-C12. These low molecular weight components with significantly higher half-lives may be due to biodegradation processes during WAF generation (Wade et al., 2017). The M5 experiment used 4 hours of mixing time in the generation of WAF instead of 12 hours in previous studies. While the impact of biodegradation before Day 0 was not fully eliminated, it was still reduced to an acceptable extent.

Table 4. Odd/Even, n-C17/Pristane (17/Pr) and n-C18/Phytane (18/Py) ratios of M5 WAF and DCEWAF treatments. Surrogate oil data from Morales-McDevitt et al. (submitted).

	Days	Odd/Even	17/Pr	18/Py
WAF	0	0.99±0.18	1.88±0.09	2.52±0.06
	3	1.06±0.03	0.85±0.07	0.72±0.12
	15	0.98±0.40	0.51±0.06	0.26±0.03
DCEWAF	0	0.98±0.16	1.95±0.07	2.52±0.03
	3	1.06±0.01	0.84±0.07	0.90±0.13
	15	0.82±0.03	0.88±0.26	0.69±0.12
Surrogate oil	N/A	0.98	1.92	2.59

Table 4 demonstrates the diagnostic ratio of alkanes in WAF and DCEWAF treatments. The odd/even ratio, also known as CPI (Carbon Preference Index), which is calculated by the sum of odd carbon-numbered alkanes divided by the sum of even carbon-numbered alkanes, is widely used in the characterization of oil fingerprints (Rasmussen, 1976, Venkatesan, 1988). The odd/even ratio

stayed at around 1.0, which is characteristic of petroleum (Wang and Fingas, 2003).

The ratios of n-C17/Pristane and n-C18/Phytane are utilized to indicate biodegradation of oil. Pristane and phytane are classic examples of isoprenoid hydrocarbons which possess branched structures, leading to the ability to resist biodegradation compared to normal alkanes (Wade and Quinn, 1980, Turner et al., 2014). The 17/Pr and 18/Py ratios measured at Day 0 were close to those in the surrogate oil, suggesting that there was not a significant removal of alkanes in the WAF preparation, probably due to the very short mixing time. The decreasing trends of 17/Pr and 18/Py in both WAF and DCEWAF treatments indicated ongoing biodegradation at similar removal rates for aliphatic hydrocarbons.

PAHs

A total of 42 PAHs and their alkyl homologues were measured in the 3 mesocosm experiments. A list of these analytes is provided in Table 5.

Table 5. List of PAHs measured

Naphthalene
C1-to C4-Naphthalenes
Biphenyl
Acenaphthylene
Acenaphthene
Fluorene
C1- to C3-Fluorenes
Phenanthrene
Anthracene
C1- to C4-Phenanthrenes/Anthracenes
Dibenzothiophene
C1-to C4-Dibenzothiophenes
Fluoranthene
Pyrene
C1-to C3-Fluoranthenes/Pyrenes
Benzo(a)anthracene
Chrysene
C1- to C4-Chrysenes
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo(e)pyrene
Benzo(a)pyrene
Perylene
Indeno(1,2,3-c,d)pyrene
Dibenzo(a,h)anthracene
Benzo(g,h,i)perylene

Unless specified, any PAH concentration referred to below is the average concentration of the 3 replicates. The TPAH concentrations for the experiments are given in Table 6a and 6b. One data point (out of 3 replicates) at M3 CEWAF T0 was determined as an outlier at 95% confidence using the Q-test and was rejected. Similar to EOE values, TPAH is lowest in WAF treatments and highest in CEWAF treatments. Pearson's correlation coefficient (r) between EOE and TPAH in each mesocosm was calculated to be >0.9 for all experiments, suggesting that there is a significant correlation between EOE and TPAH. This is expected as PAHs are the major components of oil that

produce fluorescence. This also documents that fluorescence is a valuable screening tool for the presence of oil (Wade et al., 2011a, Wade et al., 2011b).

Table 6a. TPAH concentrations in M2-M4 experiments

	Mesocosm 2			Mesocosm 3			Mesocosm 4		
Time elapsed (in days)	WAF (μg/L)	DCEWAF (μg/L)	CEWAF (μg/L)	WAF (μg/L)	DCEWAF (μg/L)	CEWAF (μg/L)	WAF (μg/L)	DCEWAF (μg/L)	CEWAF (μg/L)
0	2.0±0.6	19.1±9.3	418.4±10.1	53.7±1.8	93.8±1.1	323.0±4.2	52.3±2.7	102.0±14.0	453.8±6.8
1	-	-	-	6.6±0.8	69.8±2.0	221.5±22.7	1.4±0.7	79.7±10.8	350.3±26.0
2	-	-	-	0.5±0.2	16.2±1.0	191.1±8.6	1.1±0.4	33.2±27.5	272.1±14.5
3	1.1±1.1	4.1±1.8	141.7±22.1	0.7±0.3	11.8±1.0	163.7±10.7	1.0±0.2	10.5±2.1	242.2±24.4
4	-	-	-	0.9±0.4	7.0±1.2	72.9±22.8	-	-	-
r	0.9971			0.9333			0.9489		

Table 6b. TPAH concentrations in M5 experiment

	Mesocosm 5	
Time elapsed (in days)	WAF (μg/L)	DCEWAF (μg/L)
0	77.9±41.5	39.5±28.8
3	5.1±4.6	3.6±0.3
15	0.2±0.05	0.4±0.08
r	0.9480	0.9359

WAF, DCEWAF, CEWAF, ± and -: same as in Table 1;

r: Pearson's correlation coefficient between EOE and TPAH values in respective mesocosm experiments

Initial PAH concentrations

The ‘initial’ PAH concentrations would be defined as the PAH concentrations at time 0, where the water samples were taken just after the water was moved from the WAF generators to the mesocosm tanks.

Fluorescence is generally more sensitive to the existence of petroleum compounds in water than gas chromatographic methods. However, in this experiment, 1-3 L of water instead of 5 to 20 ml was extracted and the volume reduced to 1 ml. PAHs in the water samples underwent a 1000-3000-fold concentration in this process and became detectable in the control mesocosms while no EOE was detected. There are low but observable PAH concentrations in the control treatments in all 3 mesocosm experiments. The highest TPAH concentration in control was 1093 ng/L, which occurred at T0 at Mesocosm 4, and it is still less than 2% of the measured TPAH concentration in corresponding WAF treatment. M4 has a generally higher background TPAH concentration than M3, possibly because the seawater used in M4 was collected from a near-shore area. The PAH concentrations in control treatments are far below those in WAF, DCEWAF and CEWAF treatments.

For the M2-M4 experiments, the TPAH concentration at T0 was in the order of WAF<DCEWAF<CEWAF. Significantly higher concentrations of TPAH were observed in CEWAF treatments in all 3 mesocosm experiments. This is expected since dispersants work by breaking oil into small droplets, increasing their surface area by lowering the oil/water interfacial tension and thus accelerating the dispersal and dissolution of oil into the water column (Gong et al., 2014a). It is worth noting that the majority of dispersed oil is not dissolved in the water phase but exists in the form of numerous tiny oil droplets. This results in heterogeneity seen between the triplicate mesocosms.

For the M5 experiment, WAF treatment showed a higher TPAH concentration than DCEWAF treatment. DCEWAF treatment was produced by a 10-fold dilution of CEWAF, which was generated in the BRT system but not used in M5 experiment. The composition of PAHs at T0 for the M2-M5 experiments are shown in Figure 6. Naphthalenes made up the most significant portion of TPAH. For the M3-M5 WAF treatment at T0, the portion of naphthalenes exceeded those in the surrogate oil. This agrees with the lower molecular weight and higher water solubility for naphthalenes. The exception occurred in M2 WAF and DCEWAF treatments where naphthalenes made up a relatively lower percentage of TPAH, probably due to loss of low molecular weight (LMW) components during WAF production.

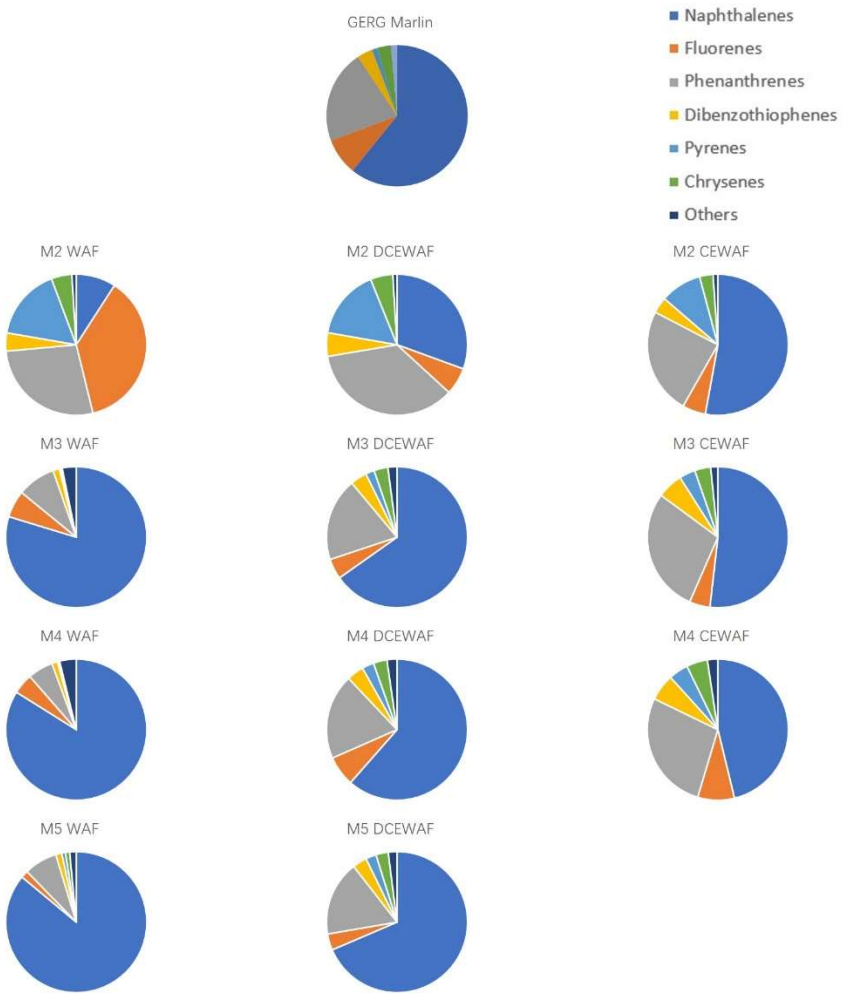


Figure 6. The composition of PAHs at T0 for M2-M5 experiments. Here 'Phenanthrenes' etc. refers to the total concentration of all phenanthrene homologues e.g. C1- to C4-phenanthrene plus phenanthrene/anthracene etc. 'GERG Marlin' refers to the analyses at GERG of the surrogate oil.

For the M3-M5 experiments at T0, the composition of PAHs of DCEWAF treatment resembled the surrogate oil (hence having lower naphthalenes concentration than in WAF treatment), while the CEWAF treatment in M3 and M4 had a lower percentage of naphthalenes in TPAH. There are less naphthalenes and more phenanthrenes and other high molecular weight (HMW) PAHs in DCEWAF and CEWAF treatments. This agrees with the observation

made by Yamada et al. (2003), where dispersion and solubilization are more effective on HMW PAHs when a dispersant is present due to their hydrophobic nature.

The total PAH concentrations increased by ~6.0 to 8.7 times with the addition of dispersants in M3 and M4, as is indicated by Table 6. For M2, the TPAH concentration in CEWAF treatment is about 209 times higher than in WAF treatment. The TPAH in M2 CEWAF treatment (418.4±10.1 ug/L) was roughly in the same range as M3 (323.0±4.2 ug/L) and M4 (453.8±6.8 ug/L), while TPAH in M2 WAF treatment (2.0±0.6 ug/L) was significantly lower than in M3 (53.7±1.8 ug/L) and M4 (52.3±2.7 ug/L). Considering the difference in the composition of PAHs in M2 WAF treatment, it is likely that part of the PAHs, especially the LMW PAHs, were lost in the generation of WAF.

In CEWAF treatments, the HMW PAH components are more enriched. Similar results were reported by Yamada et al. (2003) and Couillard et al. (2005). The degree of amplification effect can vary among individual PAHs, while an amplification factor (AF) caused by addition of dispersant for a specific PAH can be calculated by:

$$\mathrm{AF} = \frac{C_{CEWAF}}{C_{WAF}}$$

The amplification factors of naphthalene in CEWAF treatments were only ~1.45 for both M3 and M4, while the amplification factor of C2-chrysenes can reach as high as 63.15 for M3 and 237.09 for M4. This demonstrates that different PAHs can respond in a vastly different way to the addition of dispersants based on their hydrophobicity. The hydrophobicity of a specific PAH may be represented by its octanol-water partition coefficient, or K_{ow}. Figure 7 shows the plot of log K_{ow} against log AF (in part of the PAHs analyzed):

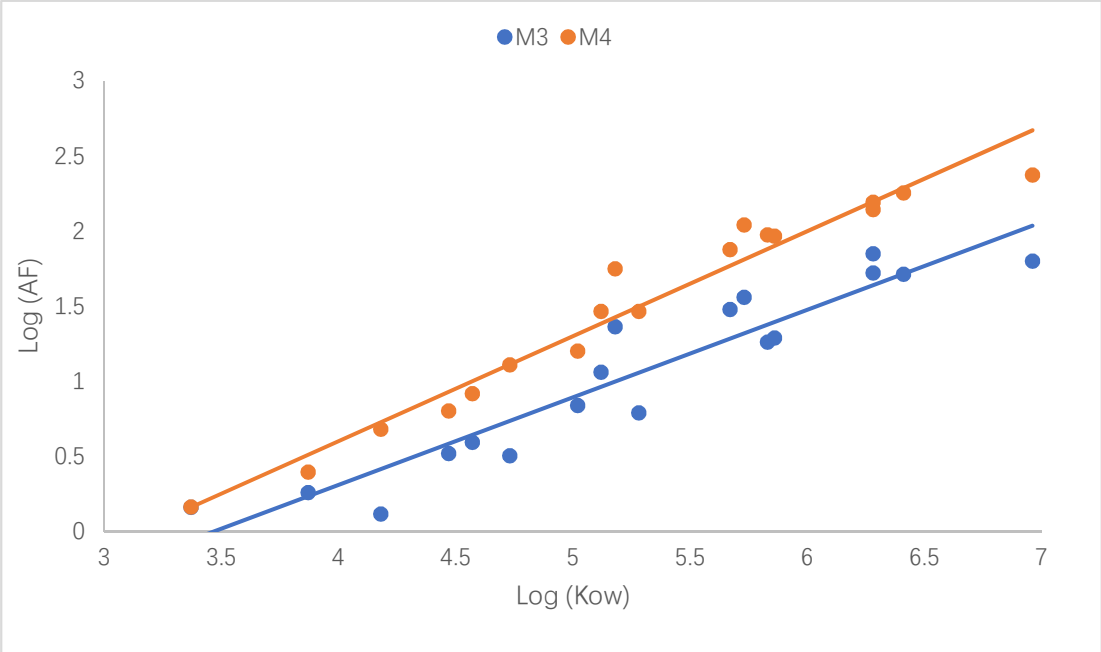


Figure 7. log K_{ow} against log AF for some PAH components analyzed. K_{ow} data after Ozretich et al. (2000).

An R^2 of 0.89 for M3 and 0.96 for M4 was calculated, suggesting that there existed a significant position relationship between log K_{ow} and Log AF, indicating that the dispersant-caused a higher amplification for HMW PAHs due to their higher hydrophobicity.

Removal of PAHs in WAF, DCEWAF and CEWAF treatments

The discussion here will be based on PAH data acquired from Mesocosm 3-5 experiments. M2 had comparatively limited data but the concentration of PAHs stayed in the range of those reported for M3 and M4. The M3 and M4 controls had low but detectable PAH concentrations representing background PAHs (Figure 8) in these samples. The controls of M4 have higher and more variable background PAH concentration compared to M3 possibly due to the fact that the seawater was collected nearer to shore where possible input sources from human activities (e.g. small spills, industrial effluents, ship operations etc.) may be present.

Figure 8. Background PAH concentrations in Control tanks

The background PAH concentrations of M3 at time 0 ranged from 173.2 to 192.0 ng/L. The all-time range of background concentration in the M3 experiment was 90.9 to 192.0 ng/L. M5 has similar background concentrations, with the range of 68.9 ng/L to 281.5 ng/L. This is in the same range of TPAH concentrations reported in Northwestern Mediterranean Sea (Guigue et al., 2014) of 8.1 to 405 ng/L. Ranges of PAHs concentrations reported for open-ocean samples are 0.56-8.80 ng/L (Berrojalbiz et al., 2011) and 5-66 ng/L (Gustafson and Dickhut, 1997). On the other hand, the initial TPAH concentration of M4 varied from 595.8 to 1379.3 ng/L. The all-time range of M4 was 433.8 to 1379.3 ng/L. This data is higher than the seawater TPAH concentrations (350-580 ng/L) observed several months after the Prestige oil spill (González et al., 2006), and comparable to polluted seawater samples (106-945 ng/L) collected from near a coastal city area (Zhou et al., 2000). Wade et al. (2016a) analyzed an extensive dataset named the Gulf Science Data, which contains analytical data for over 26,000 water samples collected pre- and post-DWH incident in the Gulf of Mexico. The median for the PAH concentration of field blanks was 56 ng/L and the mean concentration of other samples was 220 ng/L. The background PAH concentrations of M3 were all higher than the median but lower than the mean. The background PAH concentrations of M4 were all significantly higher than both mean and median. Therefore, the background PAH concentrations of M3 may reflect the condition of open ocean water in Gulf of Mexico, while the sampling of M4 may be considered as

moderately contaminated in PAHs. However, the TPAH concentrations in the control were less than 2% of the TPAH in WAF treatments (~52,300 ng/L) in M4 experiment.

Table 7. Half-lives for TPAH in M3 and M4 experiment

		Half-life (d)
M3	WAF	0.67
	DCEWAF	0.99
	CEWAF	2.11
M4	WAF	0.57
	DCEWAF	0.90
	CEWAF	3.24

The half-lives for TPAH in the M3 and M4 experiments (Table 7) indicate that ~99% total PAH in WAF tanks were removed within the first 2 days in both M3 and M4 with PAH half-lives of 0.67 and 0.57 days, respectively. DCEWAF and CEWAF samples had higher TPAH concentrations with longer half-lives. At the end of the experiment, the TPAH concentrations were 7% to 10% of the starting concentration for DCEWAF, but as much as 23%-53% TPAH still remained in CEWAF treatments (Figure 9). For the M5 experiment, 6.5% of TPAH in the WAF treatments remained after 3 days, which was higher than M3 (1.3%) and M4 (1.8%). The residual percentage of TPAH in DCEWAF treatment in M5 at day 3 was 9.2%, which is close to M3 (12.5%) and M4 (10.3%). At Day 15 of M5, both residual TPAH of WAF and DCEWAF were less than 1% of the concentration at Day 0.

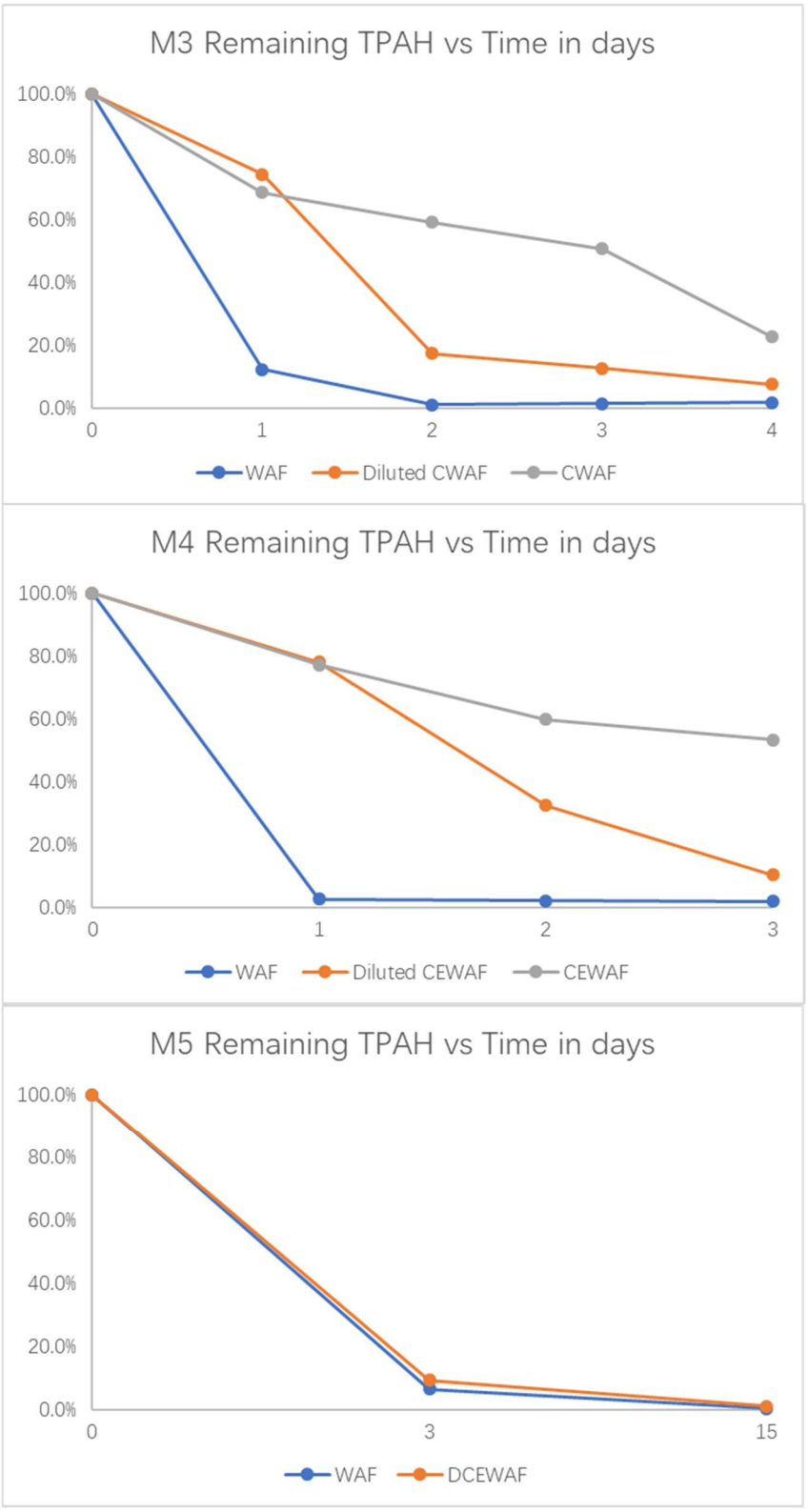


Figure 9. Remaining TPAH concentration over time in Mesocosm 3-5

Figure 10 demonstrates PAH composition changes in CEWAF treatments at the end of Mesocosms 3&4. PAH fingerprinting shows M3 and M4 had similar PAH profiles at T0, and their compositions were very close to the PAH composition in surrogate oil. At T72, the percentage of most low-molecular-weight (LMW) PAHs in TPAH remained steady, or even slightly increased. These LMW PAHs include naphthalene, fluorene and their alkylated homologues.

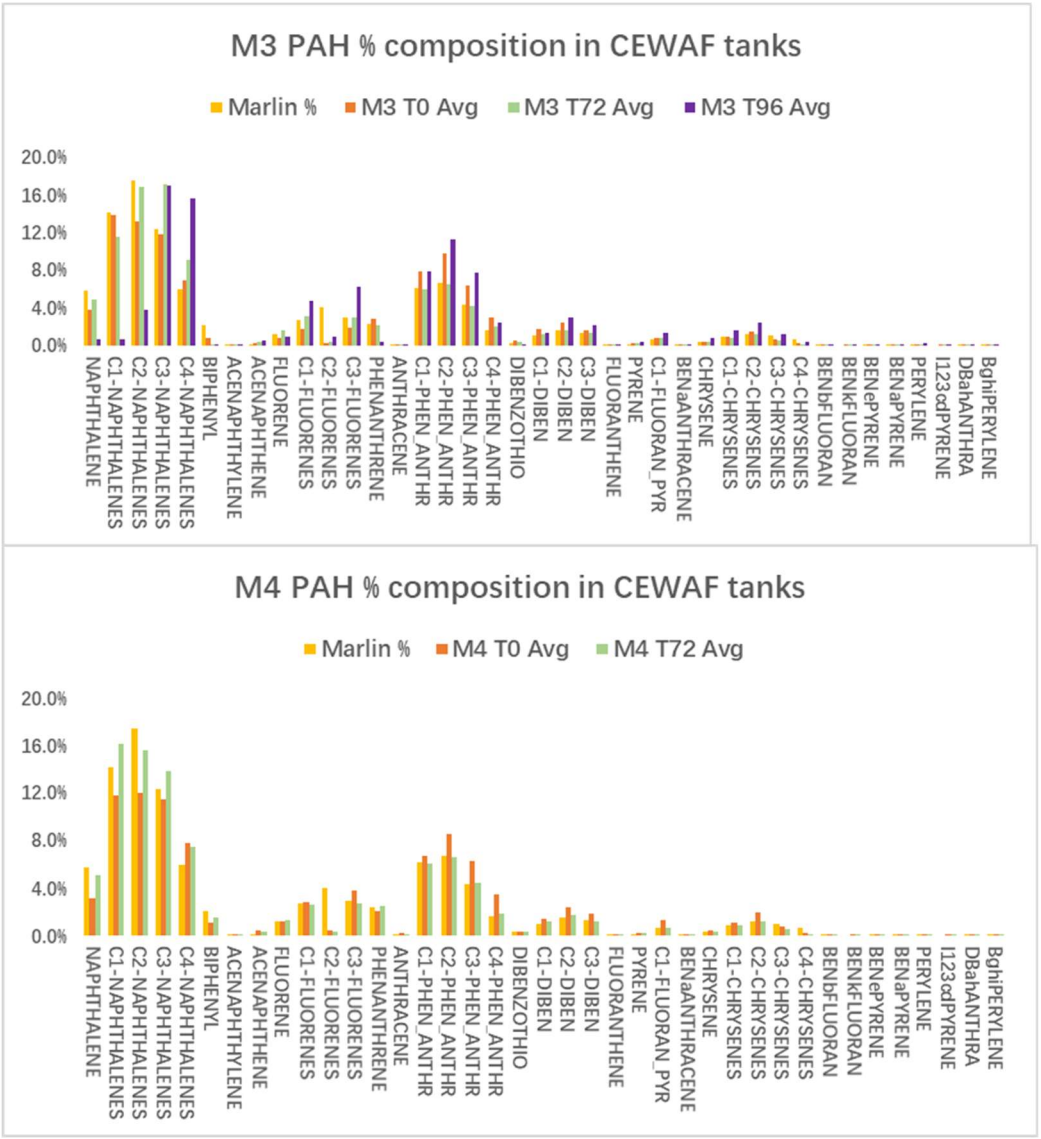


Figure 10. PAH composition changes at the end of Mesocosm 3&4

In the M3 experiment, at day 4, most of the naphthalene (99.4%), C1-naphthalenes (99.4%) and C2-naphthalenes (96.2%) were removed. At that time point, phenanthrenes became the dominant PAH species. C3- and C4-naphthalenes at day 4 stayed at roughly the same level as day 3. Chrysenes, the most abundant 4 ring PAH, followed similar trend as phenanthrenes and have slightly higher percentage in TPAH at day 4.

Figure 11 shows the progression of naphthalenes, phenanthrenes and chrysenes removed based on the percentage of their initial concentrations. A significant removal of naphthalenes and the lighter portion of phenanthrenes (Phen and C1-PHENs) can be observed in the WAF tanks within the first day and these components were almost completely removed at day 2. In comparison, C3-PHENs and chrysenes also saw a great decrease in first 2 days, but their concentrations remained stable afterwards. At day 2 in M4, an increase in chrysene concentration led to a value higher than its initial concentration. This may be due to the sample affected by heterogeneity in the mesocosm tank. On the other hand, a similar phenomenon was also observed in the mesocosm experiment conducted by Yamada et al. (2003), which may be explained by the redissolution of HMW PAHs from the surface oil slicks and suspended particles. The initial concentrations of chrysenes in WAF mesocosms were very low and these PAHs are known to easily undergo

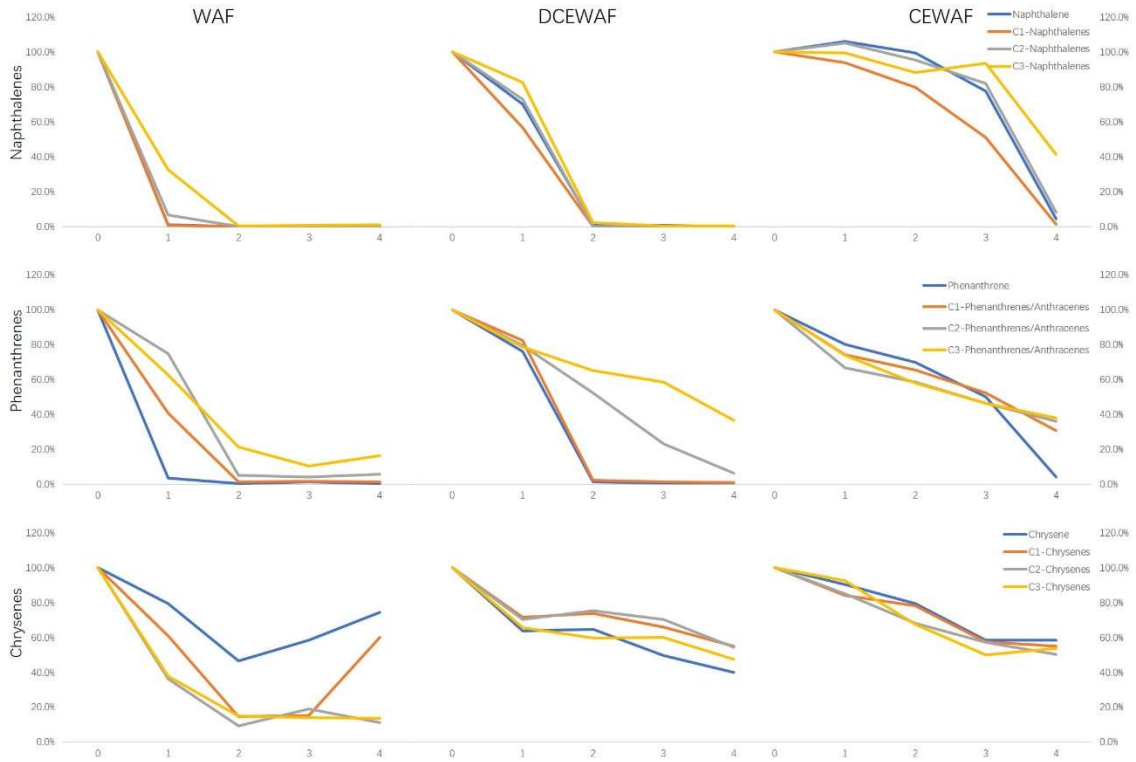
adsorption to particulates. Hence, the redissolution process, as well as the heterogeneity in sampling, may cause a fluctuation in the percentage remaining value for chrysenes. Nevertheless, in WAF mesocosms PAHs concentration dropped significantly and 99% of TPAH were gone in the first 2 days according to Fig 5&6.

In DCEWAF mesocosms, a different removal pattern was observed: naphthalenes, phenanthrene and C1-PHENs were the fastest PAHs to be removed in WAF mesocosms, but their concentrations stayed high in day 1. The removal rates greatly increased between day 1 and day 2. Similar delayed onset of the PAH degradation may be observed in CEWAF mesocosms, where removal rates significantly increased after 3 days. As high as 60%-80% of naphthalenes stayed in the mesocosms at day 3 in CEWAF treatments of both M3 and M4. The experiment of M4 ended after 3 days so there is no more information about the PAHs afterwards; however, in M3 a greater rate of decrease for naphthalenes (and phenanthrene), compared to the first 3 days, was observed on day 4.

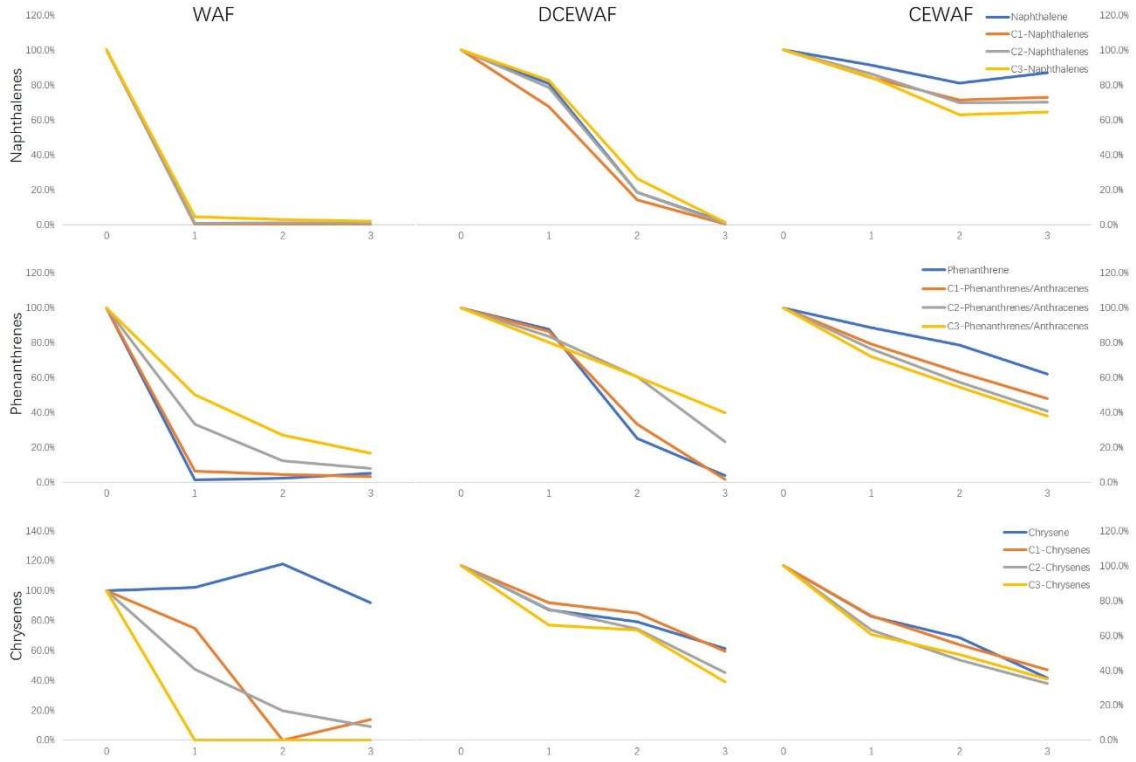
In previous studies, such a lag phase was observed in the biodegradation of oil (Campo et al., 2013, Brakstad et al., 2015), where biodegradation only took place after a few days after microorganisms were introduced to the oil-water mixture. This lag phase in the removal of PAHs is characteristic of

biodegradation (Campo et al., 2013) and could be a possible explanation for the increase of removal rates after a certain time period observed in DCEWAF and CEWAF treatments of M3 and M4. It seems that the lag phase was shorter in DCEWAF and longer in CEWAF treatment. It should be noted that during the production of the WAF and CEWAF that biodegradation of PAHs may already have started. According to the study of Campo et al. (2013), a preferential consumption of DOSS (dioctyl sodium sulfosuccinate), a component of Corexit 9500 dispersant, before PAHs began to biodegrade could be a possible explanation to this delayed degradation phenomenon. On the other hand, Doyle et al. (2018) suggested that the succession of microbial community structure may be affected by the high concentration in the CEWAF treatment in the first 72 hours. Since the early hydrocarbon-degraders may preferentially take up aliphatic hydrocarbons, this may be another cause of the lag phase.

HMW PAHs such as chrysenes in DCEWAF and CEWAF treatments had no lag phase, suggesting that there may be no biodegradation occurring of these PAHs. It was previously reported that the lag-phase for the biodegradation of 4-ring PAHs may be as long as 20 days (Brakstad et al., 2015). Four-ringed PAHs were reported to biodegrade very slowly, if at all, in water samples (Lee et al., 1978, Hinga et al., 1986). There could be a different removal mechanism regarding these HMW PAHs. Further analysis of removal rates may provide more information on the removal pattern in the mesocosms.



(a)



(b)

Figure 11. Percentage remaining of naphthalenes, phenanthrenes and chrysenes in (a) M3 and (b)M4

Rates of removal

More detailed information about the removals of PAHs can be investigated by looking into the removal rate of individual PAHs. In many previous studies, such as Campo et al. (2013), the removal of PAHs was assumed to obey the first-order rate law, i.e. the rate of removal for a specific PAH is proportional to the concentration of PAH itself. The relationship of concentration (C) can be expressed as in the equation:

$$C = C_0 e^{kt}$$

Where C_0 is the initial concentration (concentration at T0), e is the base of the natural logarithm, k is the first-order rate constant and t is the time. If natural logarithm of both sides was taken, the equation becomes:

$$\ln C = kt + \ln C_0$$

Therefore, if the natural logarithm of each PAH is plotted against time, a straight line would be expected in an ideal first-order reaction where the slope of the line would be the removal rate constant (k) for a specific PAH. The half-life ($t_{1/2}$), the time taken for a specific PAH to fall to 1/2 of its original value, can be calculated from the rate constant (k):

$$t_{1/2} = \frac{\ln 2}{k} \approx \frac{0.693}{k}$$

Actually, multiple complicated processes are involved in the removal of any PAH, such as evaporation, biodegradation, photo-oxidization and sedimentation. Precisely, the first-order rate law is used as a model to study the removal process under an ideal situation, which is rare in reality. However, one can estimate the degree of deviation from the ideal situation by calculating the R^2 of the plot.

The calculation of removal rate only applies to the data collected from the M3 and M4 experiments, since in the M2 experiment only 2 data points were collected, which is insufficient to determine the removal rate. The calculated removal rate constant and half-lives of selected PAHs in DCEWAF and CEWAF treatments of M3 and M4 are shown in Tables 8a&8b:

Table 8a. Removal rates (k), R^2 and half-life ($t_{1/2}$) in DCEWAF and CEWAF treatments of M3

	DCEWAF			CEWAF		
PAH Compounds	k	R^2	$t_{1/2}$ (d)	k	R^2	$t_{1/2}$ (d)
Naphthalene	-1.777	0.89	0.4	-0.660	0.56	1.1
C1-Naphthalenes	-2.043	0.81	0.3	-0.944	0.63	0.7
C2-Naphthalenes	-2.083	0.81	0.3	-0.527	0.57	1.3
C3-Naphthalenes	-1.686	0.91	0.4	-0.184	0.59	3.8
C4-Naphthalenes	-0.840	0.96	0.8	-0.098	0.83	7.1
Fluorene	-1.320	0.77	0.5	-0.219	0.35	3.2
C1-Fluorenes	-1.057	0.87	0.7	-0.037	0.14	18.6
C2-Fluorenes	-0.041	0.07	16.8	0.052	0.82	13.3
C3-Fluorenes	-0.145	0.31	4.8	-0.012	0.20	57.3
Phenanthrene	-1.384	0.81	0.5	-0.675	0.69	1.0
C1-Phenanthrenes/Anthracenes	-1.291	0.84	0.5	-0.271	0.94	2.6
C2-Phenanthrenes/Anthracenes	-0.675	0.91	1.0	-0.240	0.98	2.9
C3-Phenanthrenes/Anthracenes	-0.230	0.94	3.0	-0.240	0.99	2.9
C4-Phenanthrenes/Anthracenes	-0.177	0.89	3.9	-0.319	0.93	2.2
Dibenzothiophene	-1.279	0.87	0.5	-0.604	0.74	1.1
C1-Dibenzothiophenes	-0.971	0.84	0.7	-0.316	0.93	2.2
C2-Dibenzothiophenes	-0.583	0.96	1.2	-0.236	1.00	2.9
C3-Dibenzothiophenes	-0.189	0.97	3.7	-0.223	0.98	3.1
Pyrene	-0.210	0.97	3.3	-0.203	0.99	3.4
C1-Fluoranthenes/Pyrenes	-0.190	0.92	3.7	-0.190	0.99	3.6
C2-Fluoranthenes/Pyrenes	-0.122	0.91	5.7	-0.231	0.99	3.0
C3-Fluoranthenes/Pyrenes	-0.118	0.86	5.9	-0.256	0.96	2.7
Chrysene	-0.208	0.92	3.3	-0.151	0.93	4.6
C1-Chrysenes	-0.127	0.86	5.4	-0.157	0.95	4.4
C2-Chrysenes	-0.121	0.78	5.7	-0.177	0.99	3.9
C3-Chrysenes	-0.158	0.84	4.4	-0.185	0.89	3.7
C4-Chrysenes	-0.131	0.73	5.3	-0.212	0.96	3.3

Table 8b. Removal rates (k), R^2 and half-life ($t_{1/2}$) in DCEWAF and CEWAF treatments of M4

	DCEWAF			CEWAF		
PAH Compounds	k	R^2	$t_{1/2}$ (d)	k	R^2	$t_{1/2}$ (d)
Naphthalene	-1.431	0.88	0.5	-0.054	0.62	12.8
C1-Naphthalenes	-1.819	0.87	0.4	-0.111	0.85	6.2
C2-Naphthalenes	-1.654	0.85	0.4	-0.128	0.90	5.4
C3-Naphthalenes	-1.417	0.83	0.5	-0.162	0.87	4.3
C4-Naphthalenes	-0.714	0.90	1.0	-0.234	0.97	3.0
Fluorene	-1.092	0.88	0.6	-0.189	0.60	3.7
C1-Fluorenes	-1.053	0.83	0.7	-0.243	0.76	2.9
C2-Fluorenes	-0.239	0.76	2.9	-0.339	0.80	2.0
C3-Fluorenes	-0.238	0.99	2.9	-0.318	0.83	2.2
Phenanthrene	-1.094	0.89	0.6	-0.155	0.97	4.5
C1-Phenanthrenes/Anthracenes	-1.291	0.81	0.5	-0.242	1.00	2.9
C2-Phenanthrenes/Anthracenes	-0.471	0.87	1.5	-0.298	1.00	2.3
C3-Phenanthrenes/Anthracenes	-0.305	0.98	2.3	-0.318	1.00	2.2
C4-Phenanthrenes/Anthracenes	-0.460	0.99	1.5	-0.422	0.98	1.6
Dibenzothiophene	-1.265	0.87	0.5	-0.231	0.99	3.0
C1-Dibenzothiophenes	-1.096	0.86	0.6	-0.279	1.00	2.5
C2-Dibenzothiophenes	-0.504	0.90	1.4	-0.312	1.00	2.2
C3-Dibenzothiophenes	-0.298	0.99	2.3	-0.337	0.99	2.1
Pyrene	-0.273	0.98	2.5	-0.283	0.99	2.5
C1-Fluoranthenes/Pyrenes	-0.276	0.96	2.5	-0.418	1.00	1.7
C2-Fluoranthenes/Pyrenes	-0.367	1.00	1.9	-0.331	1.00	2.1
C3-Fluoranthenes/Pyrenes	-0.394	0.98	1.8	-0.364	0.98	1.9
Chrysene	-0.204	0.97	3.4	-0.330	0.97	2.1
C1-Chrysenes	-0.211	0.94	3.3	-0.298	1.00	2.3
C2-Chrysenes	-0.300	0.95	2.3	-0.368	0.99	1.9
C3-Chrysenes	-0.333	0.90	2.1	-0.337	0.97	2.1
C4-Chrysenes	-0.368	0.97	1.9	-0.425	0.99	1.6

The rate constants *k* for removal processes in Table 5 and 6 are all negative, indicating that all PAHs concentration are decreasing with time in M3 and M4 DCEWAF and CEWAF treatments. More negative values for *k* indicate faster removal rates. The coefficient of determination, or R^2, indicates how close the removal rates are compared to an ideal first-order process.

A difference can be observed in the behavior of LMW and HMW PAHs. In DCEWAF treatments of both mesocosm experiments, naphthalenes were removed the most quickly among all PAHs and their half-lives were less than 1 day. For naphthalenes, phenanthrenes and dibenzothiophenes, removal rates decreased as the degrees of alkylation increased on these PAHs. It is known that alkylated PAHs are more difficult for microorganisms to degrade (Seo et al., 2009). Biodegradation works slower on more highly alkylated PAHs (Fedorak and Westlake, 1981, Wang et al., 1998). Therefore, the longer half-lives on alkylated LMW PAHs may be indicating that biodegradation was taking place for these species. For pyrenes and chrysenes, however, their removal rates were in a reverse order: the more alkylated groups there were on these PAHs, higher removal rates were observed, especially on M4 DCEWAF treatment. Considering the low biodegradation rate of 4-ring PAHs in aqueous solution (Lee et al., 1978, Hinga et al., 1986), these PAHs had possible additional removal processes in addition to biodegradation, such as sedimentation.

For the M3 CEWAF treatment, most of the PAHs (phenanthrenes, pyrenes and chrysenes) were removed by processes other than biodegradation as their half-lives were shorter as more alkyl groups were attached to the PAHs within the same PAH family. The naphthalenes family had the shortest half-life on C1-naphthalenes PAH and longest for C4-naphthalenes. They had significant lower R^2 values (0.56-0.83), probably due to the fact that they were the first PAHs to be biodegraded. For other PAHs, R^2 value stayed high for alkylated species but lower for parent PAHs. The co-existence of both biodegradation and the other removal mechanism could have made the removal of naphthalenes deviate from the ideal first-order kinetics, hence the low R^2 value. The lower R^2 value could also have resulted from a lag phase, as reported in other studies (Campo et al., 2013).

The M4 CEWAF treatment is different from M3 because the M4 experiment was ended after 3 days. The removal rates of naphthalenes in M4 CEWAF treatment were very different from the DCEWAF treatment; like other PAHs such as phenanthrenes and chrysenes, the removal rates were fastest for the most alkylated one (C4-naphthalenes) and lowest for the unsubstituted naphthalene. In fact, the naphthalenes family had the longest half-lives and thus appeared as the most removal-resistant PAHs in the M4 CEWAF treatment, which is quite contradictory to the regular impression that naphthalenes are

usually the first to be removed in the weathering process of PAHs. This suggests that there existed ways to remove PAHs other than biodegradation.

Table 9. Half-lives (in days) of major PAHs in WAF and DCEWAF treatments of M5. Calculated based on Day 3 concentrations.

PAH Compounds	Half-life (WAF)	Half-life (DCEWAF)
Naphthalene	0.36	0.37
C1-Naphthalenes	0.26	0.28
C2-Naphthalenes	0.40	0.41
C3-Naphthalenes	0.76	0.67
C4-Naphthalenes	1.63	1.35
Biphenyl	0.56	0.57
Acenaphthylene	0.89	0.73
Acenaphthene	2.81	0.87
Fluorene	2.71	1.06
Phenanthrene	0.47	0.43
Anthracene	0.75	0.32
C1-Phenanthrenes/Anthracenes	0.98	0.84
C2-Phenanthrenes/Anthracenes	3.01	1.89
C3-Phenanthrenes/Anthracenes	3.37	2.28
C4-Phenanthrenes/Anthracenes	2.32	2.07
Dibenzothiophene	0.63	0.68
C1-Dibenzothiophenes	1.03	0.91
C2-Dibenzothiophenes	2.57	1.88
C3-Dibenzothiophenes	3.07	2.08
Fluoranthene	7.54	2.86
Pyrene	2.92	1.98
C1-Fluoranthenes/Pyrenes	3.59	1.83
C2-Fluoranthenes/Pyrenes	3.91	1.75
C3-Fluoranthenes/Pyrenes	3.21	1.79
Benzo(a)anthracene	2.48	1.63
Chrysene	14.05	3.35
C1-Chrysenes	2.99	2.22
C2-Chrysenes	2.47	1.90
C3-Chrysenes	1.94	1.77
C4-Chrysenes	2.54	1.93
Total PAHs	0.76	0.87

Table 9 demonstrates the half-lives of major PAHs in the two treatments in the M5 experiment. WAF treatment has a slightly shorter half-life for TPAH because it had shorter half-lives for naphthalene and C1-naphthalenes, which made up >60% of the total PAH concentration at Day 0. Apart from these 2 species, the half-lives of other PAHs were all longer in the WAF treatment. For two-ring and three-ring species including naphthalenes, phenanthrenes and dibenzothiophenes, their half-lives increased as the compounds became more heavily alkylated. However, in DCEWAF treatment, the four-ring components (fluoranthenes/pyrenes and chrysenes) had their half-lives in reversed order, e.g. more heavily alkylated compounds had shorter half-lives. This observation is in agreement with M3 and M4 and more likely to be related to abiotic removal of PAHs, such as the case in photo-oxidation (Kochany and Maguire, 1994) or sedimentation (Wirth et al., 2018) of heavy molecular weight (HMW) PAHs, considering the fact that four-ringed PAHs were reported to biodegrade very slowly, if at all, in water samples (Lee et al., 1978, Hinga et al., 1986).

Photo-oxidation of PAHs is a non-biotic process and follows first-order kinetics (Gong et al., 2015). The photo-sensitivity for PAHs increases with the number of rings; more alkylated PAHs are more photo-sensitive (Kochany and Maguire, 1994, National Research Council, 2005). Photo-oxidation of PAHs requires little activation as the process may begin within several minutes of the

oil being added to seawater (Hinga et al., 1986). Photo-oxidation was found to be the major pathway to remove HMW PAHs like benzo(a)pyrene from surface oil slicks and surface waters in the environment (Lee et al., 1978). However, it is difficult to assess the impact of photo-oxidation in this experiment and it could be minor due to the mesocosms not being exposed to direct sunlight.

Adsorption of PAHs to particulate matter followed by sedimentation may be another route to remove PAHs from seawater in the experiment. This process is a widespread phenomenon and responsible for downward transport of PAHs to the seafloor (Adhikari et al., 2016). LMW PAHs have higher solubility in water and they have lower tendency to partition into particulate phases. For instance, 0% of naphthalene and 0%-2% of phenanthrene were found to enter the sediments in a mesocosm experiment, while for 4-ring PAHs the percentage was 10%-94% (Yamada et al., 2003).

There are two possible pathways in which PAHs may be transferred to particulate phases and form MOS: 1) direct scavenging of oil droplets by microbes and 2) physical adsorption of PAHs to the cell surface/cell matrix (Wirth et al., 2018). The mechanism is very complicated and depends on various factors such as the concentration gradient between water and cells, physiochemical properties of the PAHs and the density and specific surface area of the phytoplankton species involved (Del Vento and Dachs, 2002).

Generally, scavenging of oil droplets favors HMW PAHs because of their lower water solubility and the tendency to concentrate in small droplets (Wirth et al., 2018). On the other hand, the adsorption of PAHs, which favors LMW PAHs due to their relative abundance in the dissolved phase, occurs relatively slower with response times of several hours to days (Dachs et al., 1999). It was demonstrated that the microorganisms produced more EPS with petroleum hydrocarbons present, which is capable of carrying these substance towards depth; the EPS produced when dispersant is present are more hydrophobic (Xu et al., 2018). Therefore, preferential removal of HMW PAHs by sedimentation process could be possible in the time period of 3-4 days of the mesocosm experiment.

In the Mesocosm 5 experiment, the individual half-lives of both PAHs (Table 9) and n-alkanes (Table 3) were measured, which provided an opportunity of comparing the removal rate of these two kinds of the major oil components. To assess the impact of dispersant on the half-life of hydrocarbons, the ratio of the half-lives (r) of a specific hydrocarbon may be calculated in the following way:

$$\mathrm{r} = \frac{t_{1/2}[DCEWAF]}{t_{1/2}[WAF]}$$

r<1 indicates that the compound has a shorter half-life in DCEWAF treatment where dispersant is present, and vice versa for WAF. The impact of

dispersants upon PAHs and n-alkanes with the same carbon atoms is shown in Table 10:

Table 10. Comparison of r-values between major PAHs and n-alkanes with the same number of carbon atoms

PAH	Alkane	r_{PAH}	r_{Alkane}
Naphthalene	n-C10	1.03	0.61
Phenanthrene	n-C14	0.91	1.47
Dibenzothiophene	n-C14	1.08	1.47
Pyrene	n-C16	0.68	1.34
Chrysene	n-C18	0.24	1.40

For n-alkanes except n-C10, the addition of dispersants increased their half-lives in seawater. For LMW PAHs such as naphthalene, phenanthrene and dibenzothiophene, the addition of dispersants had no significant impact on the half-lives; For HMW PAHs such as pyrene and chrysene, the addition of dispersants accelerated the removal of these PAHs from the water column. This may be indicative that 1) addition of dispersants indeed increased the removal rate of HMW PAHs, and 2) the removal rates of the biodegradable LMW PAHs were not significantly enhanced by the addition of dispersants. Therefore, addition of dispersants did not seem to enhance the biodegradation process, at least for the LMW PAH species.

The mechanism of PAHs removal in the mesocosm experiments were complicated. The only aspect that is certain, is that multiple processes were taking place at the same time. Abiotic processes like sedimentation may be an important way to remove PAHs as well as biodegradation for DCEWAF and CEWAF treatments.

Ratio of C2-DBTs to C2-PHENs and C3-DBTs to C3-PHENs

Alkylated PAH ratios of C2-dibenzothiophenes (DBTs)/C2-phenanthrenes (D2/P2), and C3-DBTs/C3-phenanthrenes (D3/P3) are used as indicators of biodegradation (Olson et al., 2017). Each pair of alkylated DBT/phenanthrenes have similar molecular weights, and DBT homologues are known to undergo certain biotransformation pathways (Seo et al., 2009). A significant change in this ratio, whether positive or negative, indicates microbial degradation. The D2/P2 value for the surrogate oil was 0.19 and the D3/P3 value for the surrogate oil was 0.23. The changes of these two ratios with time are shown in Figure 12.

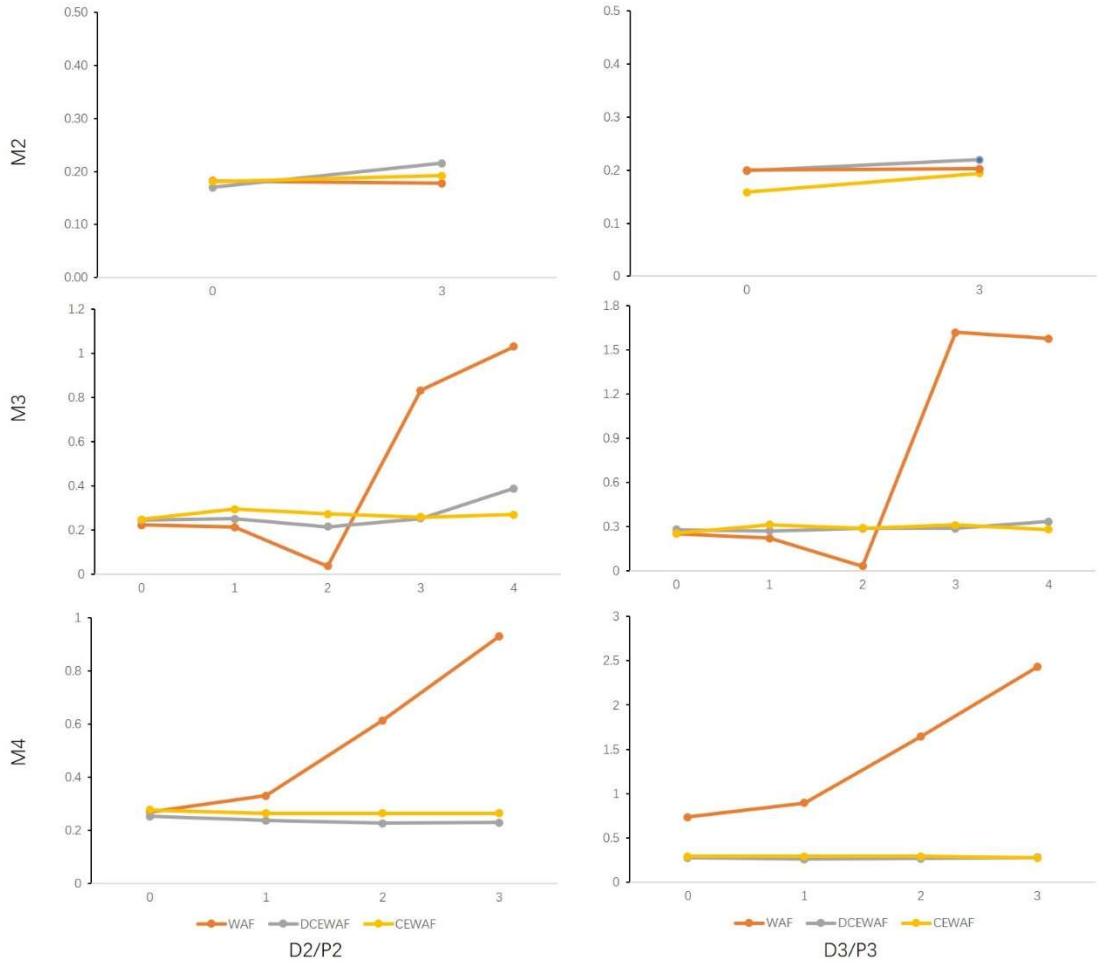


Figure 12. Changes on C2-DBTs/C2-PHENs and C3-DBTs/C3-PHENs ratios for Mesocosm 2,3 and 4 experiments.

For CEWAF treatments in M3 and M4 experiments, the D2/P2 ratio stayed at an almost constant level of 0.25-0.29 and D3/P3 at 0.25-0.31. In WAF treatments, M3 showed a rapid increase beyond day 2, while M4 showed a relatively steady increase suggesting phenanthrenes are degraded faster than dibenzothiophenes (Douglas et al., 1996). The ratios in DCEWAF was similar to CEWAF – which stayed close to a constant – in the first 3 days of M3 (0.21-0.28) and in M4 (0.22-0.28). However, at day 4 of M3 had a slight increase (0.39 for D2/P2 and 0.33 for D3/P3) similar to observations described by Olson

et al. (2017). This indicates the biodegradation of petroleum aromatic hydrocarbons is occurring in the presence and absence of Corexit.

Biomarkers

Biomarkers are organic compounds with carbon skeletons that can be related to their biogenic precursors. They are usually more resistant to degradation than n-alkanes and isoprenoid hydrocarbons, and can be useful in characterization of oil and studying the fate and transport of petroleum in the environment. In this study, tricyclic terpanes, steranes and triaromatic steroids (TAS) are the primary biomarkers to be evaluated.

After the M5 experiment, several forensic recalcitrant biomarker compound ratios were measured for selected samples including Day 0 and Day 3 samples from WAF Tank A and DCEWAF Tank A, and Day 15 samples from WAF Tank D and DCEWAF Tank D. Table 11 shows a complete list of biomarkers measured.

Table 11. List of biomarkers measured in Mesocosm 5 experiment

Abbreviation	Compound full name
C28TT	C28 extended tricyclic terpane (S)
C28TT	C28 extended tricyclic terpane (R)
C29TT	C29 extended tricyclic terpane (S)
C29TT	C29 extended tricyclic terpane (R)
Ts	Ts 18α(H)-trisnorhopane
Tm	Tm 17α(H)-trisnorhopane
BNH	17α(H), 21β(H)-28,30-bisnorhopane
25-Norhop	17α(H),21β(H)-25-norhopane
C29Tm	C29 Tm 17α(H)21β(H)-norhopane
C29Ts	C29 Ts 18α(H)-norneohopane
Diahop	15α-methyl-17α(H)-27-norhopane (diahopane)
Normor	17β(H),21a(H)-normoretane
Hopane	17α(H),21β(H)-hopane
Moreta	17β(H),21a(H)-moretane
C31HH	C31 22S 17α(H) homohopane
C31HH	C31 22R 17α(H) homohopane
Gam	Gammacerane
C32HH	C32 22S 17α(H) bishomohopane
C32HH	C32 22R 17α(H) bishomohopane
C33HH	C33 22S 17α(H) trishomohopane
C33HH	C33 22R 17α(H) trishomohopane
C34HH	C34 22R 17α(H) extended hopane
C34HH	C34 22S 17α(H) extended hopane
C35HH	C35 22S 17α(H) extended hopane
C35HH	C35 22R 17α(H) extended hopane
C27DiaS	C27 20S 13β 17α-diacholestane
C27DiaR	C27 20R 13β 17α-diacholestane
C27aaaS	C27 20S 5α,14α,17α-cholestane
C29DiaS	C29 20S 13β,17α-diaethylcholestane
C27aaaR	C27 20R 5α,14α,17α-cholestane
C29DiaR	C29 20R 13β,17α-diaethylcholestane
C28aaaS	C28 20S 5α,14α,17α,24-methylcholestane
C28abbS	C28 20S 5α,14β,17β,24-methylcholestane
C28abbR	C28 20R 5α,14β,17β,24-methylcholestane
C28aaaR	C28 20R 5α,14α,17α,24-methylcholestane
C29aaaS	C29 20S 5α,14α,17α,24-ethylcholestane
C29abbR	C29 20R 5α,14β,17β,24-ethylcholestane
C29abbS	C29 20S 5α,14β,17β,24-ethylcholestane
C29aaaR	C29 20R 5α,14α,17α,24-ethylcholestane
C27abbR	C27 20R 5α,14β,17β-cholestane

Table 11. Continued

Abbreviation	Compound full name
C27abbS	C27 20S 5α,14β,17β-cholestane
C28abbR	C28 20R 5α,14β,17β,24-methylcholestane
C28abbS	C28 20S 5α,14β,17β,24-methylcholestane
C29abbR	C29 20R 5α,14β,17β,24-ethylcholestane
C29abbS	C29 20S 5α,14β,17β,24-ethylcholestane
C20TA	C20-Triaromatic Steroids
C21TA	C21-Triaromatic Steroids
C26TA20S	C26,20S-Triaromatic Steroids
C26+27TA20R	C2620R + C27,20S-Triaromatic Steroids
C28TA20S	C28,20S-Triaromatic Steroids
C27TAR	C27,20R-Triaromatic Steroids
C28TA20R	C28,20R-Triaromatic Steroids

These oil biomarker compound ratios are useful for selective oil source characterization and identification (Wang et al., 2007). 17α(H), 21β(H)-hopane (referred to as hopane from now on) has proven to be remarkably recalcitrant against biodegradation (Prince et al., 1994) and photo-oxidation (Garrett et al., 1998). Although hopane was shown to be sometimes non-conservative in the long term (Huesemann et al., 2003), it is considered to be stable in the time span of 15 days of the mesocosm experiments and can be used to normalize PAH concentrations to investigate their biodegradation. The result is demonstrated in Figure 13.

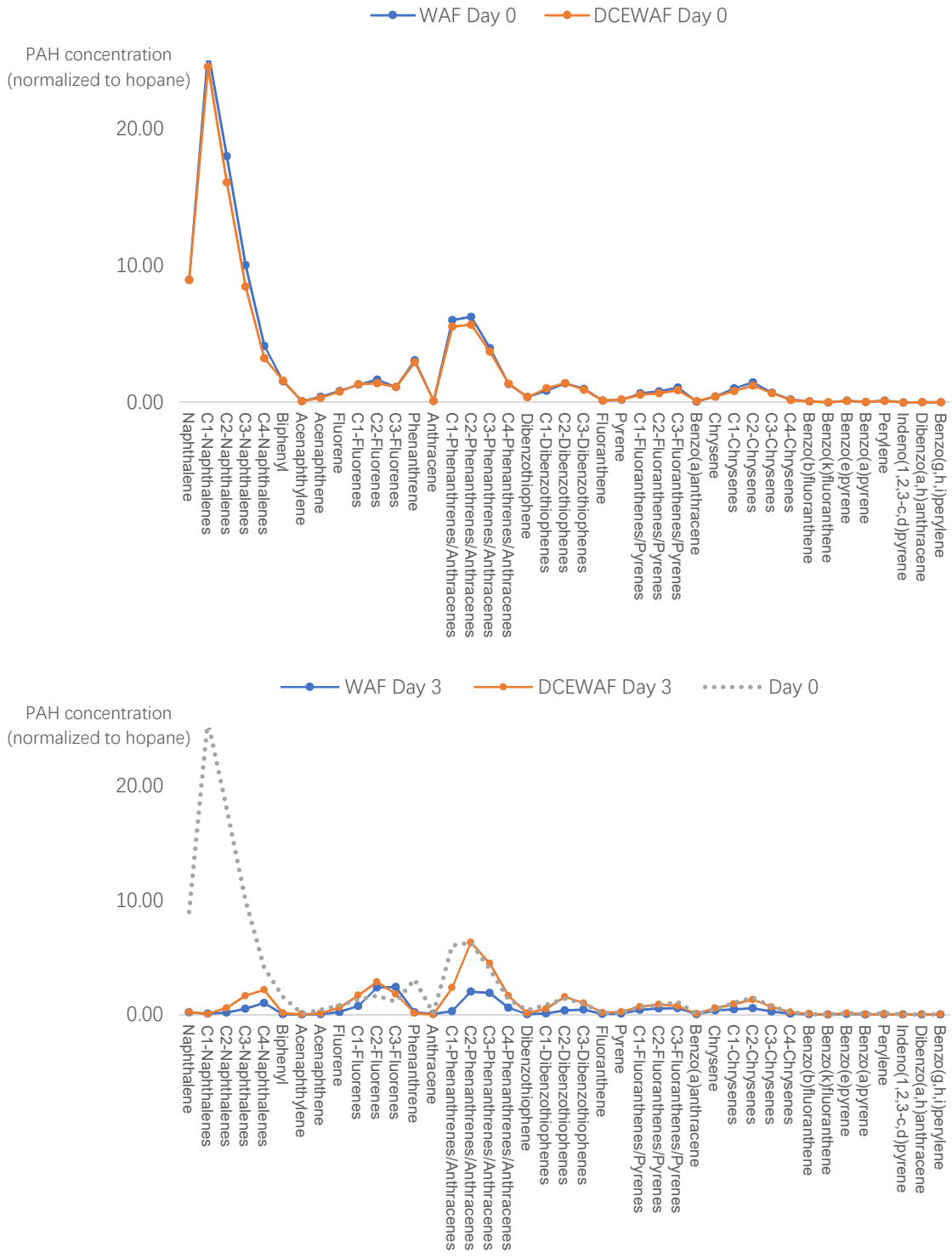


Figure 13. Hopane-normalized PAH concentrations for selected samples from M5

Almost identical fingerprinting patterns existed between WAF and DCEWAF samples from Day 0. This is probably due to the short mixing time in preparing the WAF and DCEWAF; it also suggests that addition of dispersants in the WAF preparation stage does not alter the solubility of hopane. Both WAF and DCEWAF showed a drastical decrease in the normalized concentration of naphthalenes. At Day 3, phenanthrenes also underwent a notable removal in WAF tank; however, in DCEWAF tank, C0-C1 phenanthrenes only exhibited removal to a lesser extent and C3-C4 phenanthrenes stayed roughly the same level as Day 0. Other HMW PAHs such as pyrenes and chrysenes showed similar trends in that less removal was observed in DCEWAF tank. Considering their half-lives were shorter as the PAHs became more heavily alkylated as shown in Table 9, It seems that biodegradation rates were lower in the DCEWAF tank for these HMW PAHs, and biodegradation was not the major way to remove them.

Table 12. Diagnostic ratios for selected biomarkers

	22S/(22S+22R)[a]	αβ/(αβ+βα)[b]	Ts/Tm	20S/(20S+20R)[c]	ββ/(ββ+αα)[d]
WAF A D0	0.52	0.90	0.98	0.76	0.57
DCEWAF A D0	0.56	0.90	1.08	0.73	0.57
WAF A D3	0.59	0.90	0.95	0.69	0.51
DCEWAF A D3	0.56	0.91	1.06	0.66	0.57

Table 12. Continued

	22S/(22S+22R)[a]	αβ/(αβ+βα)[b]	Ts/Tm	20S/(20S+20R)[c]	ββ/(ββ+αα)[d]
WAF D D15	0.55	0.83	1.06	0.74	0.48
DCEWAF D15	0.52	0.87	1.02	0.81	0.53

a. 22S/(22S+22R) for C31-17α(H) homohopane

b. 17α(H),21β(H)-hopane/[17α(H),21β(H)-hopane+17β(H),21α(H)-moretane] for C30-triterpanes

c. 20S/(20S+20R) for C29-5α(H),14α(H),17α(H)-steranes

d. 5α(H),14α(H),17α(H)/[5α(H),14α(H),17α(H)+ 5α(H),14β(H),17β(H)] for C29-steranes

Very close ratios for select biomarkers were observed during the 15-day experiment, as is shown in Table 12. These ratios were selected due to their wide applications: the ratios of 20S/(20S+20R) and ββ/(ββ+αα) for the C29-steranes are used as maturity parameters (Mackenzie et al., 1980) and source tracers of weathered oil residues (Wang et al., 1994); the ratios of 22S/(22S+22R) and αβ/(αβ+βα) for C30-triterpanes are also maturity parameters (Mackenzie, 1984) and applied in source matching (Kvenvolden et al., 1995). The similarity of these parameters suggested that the weathering processes in the mesocosms did not alter most of the biomarker parameters and these molecular parameters were good tracking indices of weathered oil in the experiment.

For samples taken at Day 15, most PAH concentrations were reduced to barely above detection limit. However, the recalcitrant biomarkers stayed in the system and can be measured. The terpane and sterane ratio to hopane shown in Figure 14 suggested that these biomarkers stayed remarkably similar to the original oil. No major degradation of these biomarkers was observed after 15 days. On the other hand, the low molecular weight triaromatic steroids (C20TA and C21TA) were found to slightly decrease at Day 15 in the WAF treatment, and significant depletion of TAS was observed in DCEWAF treatment.

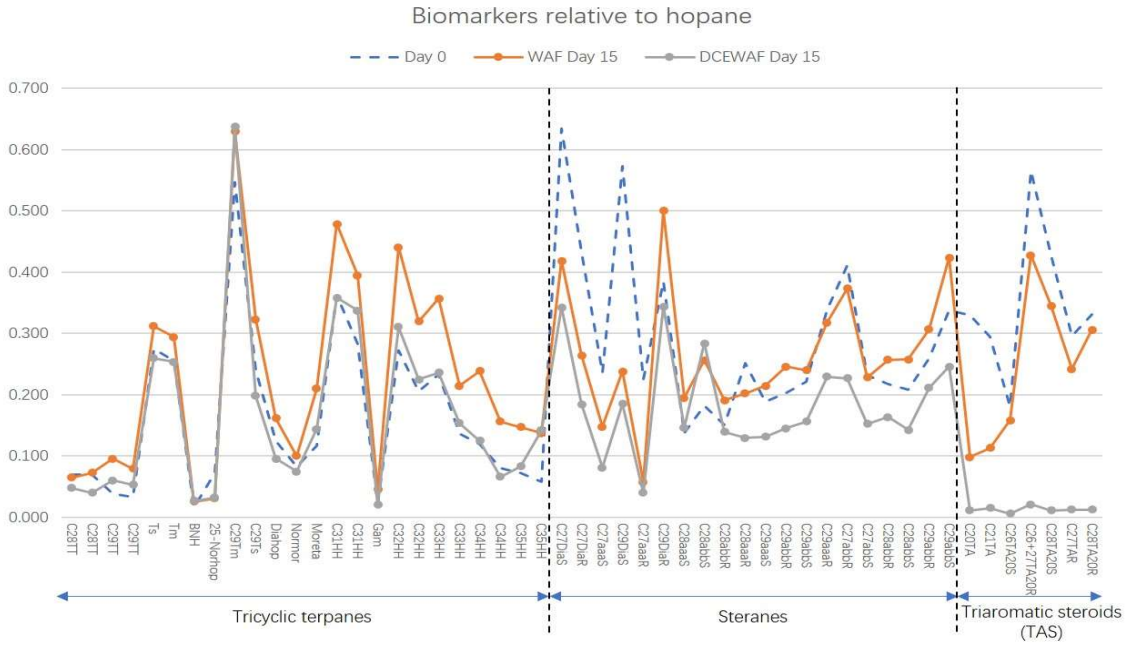


Figure 14. Concentrations of biomarkers (normalized to hopane concentrations).

Normally, TAS is considered more stable than regular steranes and hopanes in terms of biodegradation (Wenger et al., 2002). In previous studies,

this phenomena of TAS depletion was considered as a sign for the existence of photo-oxidation (Aeppli et al., 2014, Radović et al., 2014). However, the mesocosm in this study was not exposed to direct sunlight and the lamp used as light source in this experiment was unlikely to cause major photo-oxidation. Supporting evidence for this can be found in Wozniak et al. (2019). The depletion of TAS was not found in the WAF treatment either and there is little evidence on dispersants enhancing the photochemical sensitivity of aromatic biomarkers. Therefore, photo-oxidation seems unlikely to be the cause of TAS depletion observed here. Further investigation may be required to understand the mechanics behind the observed TAS depletion.

CONCLUSIONS

This study utilized baffled recirculation tanks (BRTs), compared to the traditional CROSERF method, to generate large amounts of WAF and CEWAF. The heterogeneity due to the hydrophobicity of petroleum components was the cause of the variability seen in the experiments. Nevertheless, this BRT system was shown to be capable of generating large amounts of WAF and CEWAF required for the mesocosm experiments with adequate reproducibility.

PAHs were seen in all treatments including control, WAF, DCEWAF and CEWAF. The concentrations of PAHs in control treatments suggested that a PAH background exists in the studied area where seawater was collected. Nevertheless, the PAH content in control tanks was low compared to those in other treatments.

Based on the data acquired in the 4 experiments (M2-M5), the questions raised in the earlier part of the dissertation are ready to be answered:

What are the initial PAH concentrations in mesocosms?

The initial concentrations of TPAH in WAF treatments was around 2 mg/L. The addition of dispersant in DCEWAF and CEWAF treatments was found to

greatly increase the initial concentration of oil, represented by estimated oil equivalents (EOE), and total PAHs (TPAH) in corresponding treatments in comparison to WAF. The use of dispersant in these mesocosms caused a ~6.0 to 8.7 times increase in TPAH. Different PAH species reacted differently to the addition of dispersant. The amplification factor (AF) for each individual PAH was calculated, and it was found that the AF of PAHs was related to their hydrophobicity, represented by its octanol-water partition coefficient (K_{ow}). More hydrophobic components such as chrysenes had significantly higher dispersant-caused amplification in these experiments.

How does PAH concentration in WAF, DCEWAF and CEWAF tanks change over time respectively?

Removal of PAHs was observed to occur across all treatments including WAF, DCEWAF and CEWAF within the time span of the mesocosm experiments. WAF treatments had the fastest PAH removal rate: ~99% of TPAH were removed in the first 24 hours. DCEWAF and CEWAF had higher initial concentrations and relatively lower removal rates. The rate of removal on individual PAHs was investigated. The removal pattern of alkylated PAHs, especially HMW ones, suggested that abiotic processes may be an important cause of PAH removal. An analysis of the ratio of C2-DBTs to C2-PHENs

(D2/P2) and C3-DBTs to C3-PHENs (D3/P3) showed similar results with Olson et al. (2017).

In the Mesocosm 5 experiment, an improved WAF preparation method allowed us to generate WAF and DCEWAF in similar concentration in terms of EOE content. Both WAF and DCEWAF had similar half-lives for EOE and the standard deviation of EOE concentrations for the two treatments over the 15-day period were mostly overlapped, suggesting that dispersant made no significant difference on the removal of oil. It was shown that PAHs higher than phenanthrene had higher removal rates in DCEWAF treatment. According to the biomarkers data, there was not enough evidence that biodegradation played a significant role in the removal of these HMW PAHs. Processes other than biodegradation, such as sedimentation, may be associated with dispersants on the lowering of half-lives for these PAHs.

What is the cause of PAH removal? Which factor is more importantant in PAH removal, biodegradation or sedimentation?

Several recalcitrant biomarkers were measured for selected samples from the M5 experiment. Hopane was selected as a standard to normalize PAHs to determine the extent of their biodegradation and that of other biomarkers due to its stability and resistance to biodegradation. It was shown that the hopane

concentration in mesocosms was unaffected by the existence of dispersants. It was found that the low molecular weight PAHs such as naphthalenes and C0-C2 phenanthrenes in both WAF and DCEWAF treatments were subject to biodegradation, based on their hopane-normalized concentrations. However, the normalized concentrations of HMW PAHs in the DCEWAF treatment suggested that their biodegradation rate was lower than in WAF treatment and their major removal pathway was not biodegradation. The removal rates of these HMW PAHs in M3-M5 experiments also showed that the more alkylated species had lower half-lives, which is the opposite of biodegradation, where less alkylated species usually have lower half-lives. These suggested that sedimentation may be an important approach to remove PAHs from the water column. This provides additional evidence towards hypothesis made by Passow et al. (2017) that Corexit addition may lead to increased sedimentation rate of oil, and that Corexit and EPS components regulate petroleum hydrocarbon distribution between the water column and sinking MOS (Xu et al., 2018).

Diagnostic ratios of certain biomarkers were calculated and there was no significant change in these ratios throughout the 15-day experiment period. However, triaromatic steroids (TAS) depletion was observed in the DCEWAF treatment. This was considered a sign of photo-oxidation in previous studies, but it was not favored here by the experimental conditions, and TAS depletion

was not observed in the WAF treatment either. Therefore, it was considered unlikely to be evidence of photo-oxidation. Further studies are required to have a detailed insight regarding this phenomenon.

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